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PENETRATION AND INVASION OF CERATOCYSTIS PICEAE IN WHITE OAK WOOD [1]

CHARLES L. WILSON [2]

(WITH 13 FIGURES)

Ceratocystis piceae (Münch) Bakshi causes a stain in the wood adjacent to the galleries of the ambrosia beetle *Corthylus columbianus* Hopk. in white oak (Wilson, **1959**). This paper is a description of the mode of penetration and invasion of *C. piceae* in the stained wood accompanying the galleries. Most of the histological work published to date on the growth of staining fungi in wood has dealt with conifers. Except for the work of Lagerberg et al. (**1927**), and Hubert (**1931**), little attention has been given the mechanism whereby species of *Ceratocystis* penetrate coniferous wood. Lagerberg et al. studied the growth of species of *Ceratocystis* in pine and spruce and found penetration to take place through pits and directly through the non-porous areas of tracheid walls. They stated that direct penetration usually takes place by the formation of an "adpressorium" from which a fine thread grows out directly through the wall. They believed that penetration resulted mainly from mechanical force and was not the direct result of enzymatic

[1] Published with the approval of the Director of the Arkansas Agricultural Experiment Station. This paper is an outgrowth of a study of the fungi associated with the Columb[illegible] Timber Beetle started while the author was a graduate student at West Virginia University.

[2] Assistant Professor in Plant Pathology. The author would like to thank Drs. J. G. Leach, A. F. Verrall, and R. P. True for their constructive criticisms of this work and Dr. John Hunt for his identification of *C. piceae*.

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action. Hubert (**1931**) illustrates and discusses appressoria and appressorium-like structures in coniferous wood, but he disagrees with Lagerberg et al. on the mode of penetration. He states that the small penetration threads are the same diameter as young hyphae and that enzymes produced at hyphal tips can dissolve the wall material necessary for passage through the cell wall.

Most of the growth of staining fungi is confined to parenchymatous cells, where there is apparently a greater supply of nutrients than in the vessels and tracheids (Münch, **1907**). In species of pine vertically arranged parenchyma is limited to the epithelial cells of resin ducts, and staining fungi spread vertically mainly within the tracheids.

MATERIALS AND METHODS

Blocks of wood including part of the gallery and adjacent stain were fixed in formalin-aceto-alcohol (FAA) for 2 days to 2 weeks and sections 8–40 μ thick were cut on a sliding microtome. Several different biological stains and treatments, that provided a variety of effects for study, were used on these sections. Soaking the sections in a 2% solution of NaOCl for 10 minutes made a good preparation for observing the hyphae in and adjacent to the galleries. The safranin-and-fast-green combination gave the best definition of the wood cells. Blocks of white-oak sapwood ($1 \times 2 \times 4$ in.) were sterilized by placing them for 24 hours in a closed vessel containing 5 ml/l of propylene oxide. The blocks were placed in Petri dishes with several layers of moistened filter paper in the bottom and inoculated with spores of *C. piceae*. After 2 weeks they were fixed in FAA and histological preparations were made as described above.

OBSERVATIONS

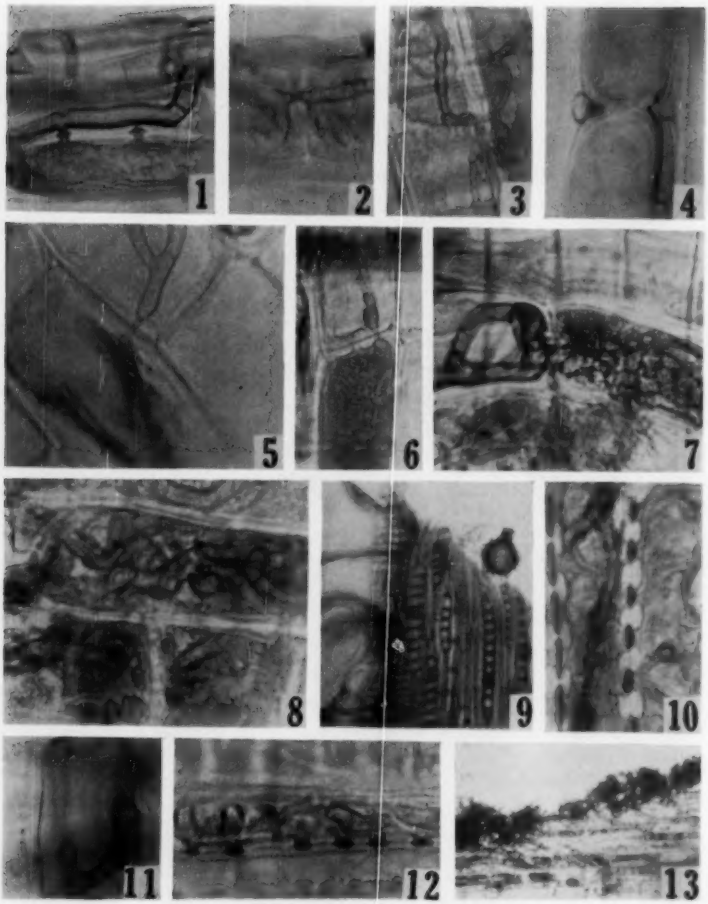
Ceratocystis piceae was isolated from the galleries of *C. columbianus* and the adjacent stained wood. Perithecia of this fungus were present in all occupied galleries examined as well as in some old galleries sealed in the wood. A yeast, *Pichia* sp., and a species of *Fusarium* were also isolated from the galleries and accompanying stained areas (Wilson, **1959**). Hyphae of *C. piceae* predominated in the galleries and stained wood adjacent to the galleries. It was possible to trace some of these hyphae to perithecia (FIG. 9), and sterile blocks of sapwood inoculated with *C. piceae* contained hyphae similar to those generally found in the galleries and adjacent stains. Most of this study was made from sections of naturally occurring galleries and stained wood; however, com-

parable conditions in relation to penetration were found when *C. piceae* was grown on sterile sapwood.

A dense growth of the mycelium of *C. piceae* lines the galleries of *C. columbianus* (FIG. 13). Although the stain may extend 6 inches or more from the gallery, it was not possible to trace the hyphae of *C. piceae* or any other fungus further than 2 inches from the gallery. The hyphae of *C. piceae* grow primarily in the vertical xylem-parenchyma and uniseriate-ray cells. Near the gallery there is some growth within the vessels (FIGS. 1, 10). The hyphae are intracellular (FIGS. 2, 3, 8) except near the gallery, where they may grow for a short distance intercellularly (FIG. 4). *C. piceae* appears to be able to penetrate white-oak cells only through pits (FIGS. 5, 11). Lagerberg et al. (**1927**) reported that this species frequently penetrates the tracheids of pine directly.

Most of the vertical spread of *C. piceae* in the wood is through the vertically arranged xylem parenchyma. The hyphae pass from one parenchymatous cell to the next through simple pit-pairs in the wall (FIG. 2). Simple pit-pairs also occur between the xylem-parenchyma and uniseriate-ray cells and through these pits hyphae enter the ray tissue. Once within a ray cell, the hyphae spread throughout all the cells of that uniseriate ray. The ray cells may become completely filled with fungus mycelium (FIG. 8), in which case the host protoplast is destroyed and the mycelium is surrounded by a brown matrix. The radial extension of the fungus usually stops where the infected ray abuts upon a vessel. This may be explained by the fact that there are no pit-pairs between vessels and uniseriate-ray parenchyma. There are half-bordered pit-pairs between the xylem-parenchyma cells and vessels and hyphae occasionally pass through these pits (FIG. 1). Since the uniseriate rays are only one cell thick, there is no possibility of tangential spread of the fungus within this tissue.

The hyphae move from one vessel to another through bordered pits (FIG. 10). Short side branches of the mycelium penetrate the bordered-pit membranes between the vessels without constricting. An area of the primary wall adjacent to the point of fungus penetration and including the torus is usually darkened by the penetration of the fungus (FIGS. 10, 12). Hyphae also pass readily without constricting through the half-bordered-pit membranes between the wood parenchyma and vessels (FIGS. 1, 12). The simple-pit membranes that occur between the living cells of individual uniseriate rays and between the vertical xylem parenchyma, and also at the point where such tissues are in contact, appear to afford more resistance to the passage of hyphae. However, most of the growth of the mycelium occurs in these tissues. This is probably



FIGS. 1–13. Growth of *C. piceae* in stained wood. FIG. 1. Hypha passing through half-bordered-pit membrane between xylem parenchyma and vessel. About 450 ×. FIG. 2. Hypha passing through simple-pit membrane between two xylem-parenchyma cells. About 500 ×. FIG. 3. Hyphal penetration of simple-pit membranes between uniseriate-ray cells. Note appressoria. About 500 ×. FIG. 4. Hypha growing intercellularly. About 600 ×. FIG. 5. Hyphal penetration of simple-pit membrane between uniseriate-ray cells. Note constriction. About 700 ×. FIG. 6. Hypha penetrating simple-pit membrane of uniseriate-ray cells. Note appressorium. About 500 ×. FIG. 7. "Intercalary appressoria." About 600 ×. FIG. 8. Coiled mass of mycelium within uniseriate ray cells. About 700 ×.

because there is a better supply of nutrients for the fungus within living parenchymatous cells.

The manner by which *C. piceae* penetrates the membrane between simple pit-pairs differs from that where the membrane between bordered and half-bordered pit-pairs is penetrated. Penetration of the membrane between simple pit-pairs usually involves the formation of appressoria. A hyphal tip comes in contact with the wall and enlarges. A thick wall is laid down just behind the terminal swelling (FIGS. 3, 6) and a small penetration peg is then sent through the pit membrane (FIG. 6). The penetration peg originates from the terminal portion of the hyphal tip where the fungus cell wall is thin. No discoloration of the host primary wall was observed in areas where the hyphae penetrate the pit membranes of living cells. Once the penetration peg enters the lumen of the adjacent cell it enlarges and forms a full-sized hypha (FIG. 5). Occasionally "intercalary appressoria" may be formed (FIG. 7). A hypha comes in contact with the cell wall and septations are laid down, forming one to several cells adjacent to the host cell wall. The fungus wall thickens except at the point where a small penetration peg protrudes through the simple-pit membrane.

Hyphae were not found to enter the large medullary rays, even in areas where adjacent parenchymatous tissue contained mycelium or where the gallery was constructed through the ray. Tannin bodies in the medullary-ray cells gave the typical tannin reaction when treated with $FeSO_4$, whereas the "tannin bodies" of tissues invaded by the fungus did not. Therefore, it appears that the large medullary rays are resistant to the invasion of *C. piceae*.

DISCUSSION

Appressoria and appressorium-like structures have generally been considered to be formed only when a fungus first penetrates the host tissue. It was evident from this study that *Ceratocystis piceae* utilizes this mechanism in its spread within the host. Lagerberg et al. (**1927**) and Hubert (**1931**) have already pointed out that this type of mechanism is formed in coniferous wood by species of *Ceratocystis* and certain stainers belonging to the Fungi Imperfecti for the direct penetration of

FIG. 9. Perithecium in gallery. About 50 ×. FIG. 10. Hypha passing between vessels. Unstained preparation. Note darkening of primary wall. About 700 ×. FIG. 11. Hypha passing through simple-pit membrane of uniseriate-ray cell. About 600 ×. FIG. 12. Hyphae passing through half-bordered-pit membranes between xylem-parenchyma cell and vessel. About 450 ×. FIG. 13. Section of gallery of *C. columbianus*. Note dense mycelial growth of *C. piceae*. About 45 ×.

tracheids. Appressoria are of widespread occurrence among different fungi and function in the penetration of external cells and stomata. They may be important also in the invasion of cells of internal tissues.

C. piceae spreads vertically in the wood through the xylem parenchyma and there is little growth within the vessels. In conifers there is inconspicuous xylem parenchyma and the vertical spread of staining fungi occurs mainly within the tracheids. This anatomical difference between conifers and hardwoods has not been previously pointed out in relation to the vertical spread of staining fungi.

The appressoria formed by *C. piceae* in parenchymatous cells appear to be adaptations for the mechanical penetration of the pit membrane. A thickening of all but a small portion of the fungus cell wall could cause considerable turgor pressure to be exerted in a localized area. The possibility of localized enzymatic activity at the point of penetration cannot be ruled out. However, since no discoloration or dissolution of the primary wall at the point of penetration was observed, a purely mechanical form of penetration of the parenchymatous cells seems more probable. Hyphae penetrated the bordered-pit membrane between vessels and the half-bordered-pit membrane between xylem parenchyma and vessels without constricting. Also, a discoloration of the primary wall was observed at the point of hyphal penetration. In these cases enzymatic activity as described by Hubert (**1931**) appears to be involved in penetration. It appears then that *C. piceae* utilizes both a mechanical and enzymatic means for penetration, depending upon the nature of the cells invaded.

It is significant that the large medullary rays were not invaded, since this tissue in other hardwoods and in conifers has been found to be extremely susceptible to attack by staining fungi (Hubert, **1931**). Simple pit-pairs were observed between xylem parenchyma and parenchymatous cells of the medullary ray and also between uniseriate-ray cells and medullary-ray cells. Therefore, the growth of *C. piceae* into medullary-ray cells does not appear to be mechanically inhibited by the absence of pits. The fact that the fungus did not enter the medullary-ray tissue even when a gallery was constructed through it indicates that the resistance of this tissue to *C. piceae* is physiological. This study was made on the growth of the fungus in living wood and in non-living medullary-ray cells this resistance may not be expressed.

SUMMARY

A study was made of the penetration and invasion of *Ceratocystis piceae* in stained white-oak wood adjacent to the galleries of the ambrosia beetle *Corthylus columbianus*.

The growth of *C. piceae* in white oak for the most part is intracellular and confined primarily to vertical xylem parenchyma and uniseriate-ray cells. The large medullary rays were not invaded.

C. piceae appears to be able to penetrate white-oak cells only through pits. Penetration of the membrane between simple pit-pairs generally involves the formation of appressoria, whereas the membrane between bordered and half-bordered pit-pairs is penetrated without the formation of such structures.

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LITERATURE CITED

Hubert, E. E. 1931. An outline of forest pathology. John Wiley & Sons, London. 531 pp.

Lagerberg, T. G., G. Lundberg, and E. Melin. 1927–28. Biological and practical researches into blueing in pine and spruce. Svenska Skogsvårdsför. Tidskr. **25**: [145]–272, [561]–739.

Münch, E. 1907. Die Blaufaule des Nadelholzes. Naturw. Ztschr. Forst. Landw. **5**: 531–573.

Wilson, C. L. 1959. The Columbian timber beetle and associated fungi in white oak. For. Sci. **5**: 114–127.

NUTRITIONAL REQUIREMENTS OF SACCHAROMYCOPSIS GUTTULATA (ROBIN) SCHIÖNNING

M. SHIFRINE[1] AND H. J. PHAFF

(WITH 1 FIGURE)

In a previous paper (Shifrine and Phaff, **1958**) a method has been described for the isolation of pure cultures of *Saccharomycopsis guttulata* from fecal matter of rabbits. In addition, its morphological, physiological and cytological characteristics, the life cycle, and its ecology have been described. The inability of this yeast to grow in most of the common media used for the cultivation of yeasts is responsible for the long delay between its first observation *in situ* by Remak (**1845**) and its isolation in pure culture by Parle (**1956**). Parle used as a basal medium yeast extract (Difco) plus glucose. To this he added an extract of the stomach contents of a rabbit which supplied the necessary growth factors. He found that the growth-promoting material was stable upon autoclaving. After precipitation of the proteins with ammonium sulfate, the dialyzed supernatant had lost its growth-promoting activity. Shifrine and Phaff (**1958**) found that an autolysate of baker's yeast also contained the growth factors. Only certain types of commercially available dehydrated autolysates were shown to be suitable. Addition of Proteose Peptone to yeast autolysate improved the medium. The purpose of the present investigation was to study in more detail the growth requirements of *S. guttulata*. It will be shown that *S. guttulata* can be grown in a defined synthetic medium as well as in certain complex protein hydrolysates. The pure culture used in these experiments has been described in detail in our previous publication (Shifrine and Phaff, **1958**). In addition a few comparative experiments were done with the culture isolated by Parle (**1956**).

EXPERIMENTAL METHODS

Inoculation and growth. Unless specified otherwise, growth was studied in test tubes (16 × 150 mm) containing 5 ml of medium, or

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in Erlenmeyer flasks (capacity 25 ml) with 5 ml of medium. When serial transfers were attempted in a certain medium, the first tube of a series received 0.1 ml inoculum from a 24-hour culture (*ca.* 10^6 cells) while subsequent transfers were done with 0.05 ml. Growth was judged visually as described by Wickerham (**1951**). A medium was considered adequate if it successfully supported growth (++ to +++) during five consecutive transfers. All cultures were incubated at 37° C.

RESULTS

Growth in natural media. During the first attempts to isolate pure cultures of *S. guttulata* it was realized that malt extract or yeast extract (Difco) plus glucose did not support continual growth. This observation confirms the results of previous workers who attempted to obtain pure cultures of *S. guttulata*. It is reasonable to assume that the highly specific habitat of this yeast in the intestinal tract of rabbits has led to unusual nutritional requirements. Evidently one or more growth factors are required by *S. guttulata*, which are not present in these media. Thus, a number of other complex yeast and protein hydrolysates were tested for their ability to support growth.

First, yeast autolysate with 2% glucose, a medium known to support growth of certain exacting bacteria, e.g. *Lactobacilli* (Snell, **1949**), was used. Yeast autolysate was prepared in the laboratory according to the procedure of Bouthilet et al. (**1949**) and was used in a concentration of 10% (v/v). This medium supported good growth of *S. guttulata*. When pure cultures had been isolated using this medium, commercially available dehydrated autolysates were substituted for the autolysate prepared in the laboratory. As noted before during our isolation studies (Shifrine and Phaff, **1958**) and by Parle (**1956**), Difco yeast extract, which is prepared from autolysed yeast, did not support growth in concentrations up to 1%. However, another commercial preparation, Albimi yeast autolysate (Albimi Lab., Inc., Brooklyn, N. Y.), in a concentration of 1%, supported good growth. Two percent glucose was added in all cases.

Next, various peptones were tried in a concentration of 1%. To supply a mineral and vitamin supplement, the peptones were dissolved in yeast carbon base (Difco). The glucose concentration was adjusted to 2%. The composition of yeast carbon base, as described by Wickerham (**1951**), is given in TABLE I. It was found that Bacto-Peptone (Difco) did not support growth, Neopeptone (Difco) supported weak to fair growth and Proteose Peptone (Difco) supported good growth.

TABLE 1

COMPOSITION OF SYNTHETIC MEDIA EMPLOYED IN THE STUDY OF NUTRITIONAL REQUIREMENTS OF SACCHAROMYCOPSIS GUTTULATA

Synthetic medium*		Yeast carbon base**		Synthetic medium (final)	
	g/100 ml		g/100 ml		g/100 ml
Glucose	1	Glucose	1	Glucose	2
				$(NH_4)_2SO_4$	0.50
	mg/100 ml		mg/100 ml		mg/100 ml
NaCl	15	NaCl	10	NaCl	10
KCl	76	KH_2PO_4	100	KH_2PO_4	100
$MgCl_2 \cdot 6H_2O$	42	$MgSO_4 \cdot 7H_2O$	50	$MgSO_4 \cdot 7H_2O$	50
Na_2SO_4	200	$CaCl_2 \cdot 2H_2O$	10	$CaCl_2 \cdot 2H_2O$	10
Na_2HPO_4	66				
$ZnCl_2$	1		µg/100 ml		µg/100 ml
$FeCl_3 \cdot 6H_2O$	2.5	H_3BO_3	50	H_3BO_3	50
$MnCl_2 \cdot 4H_2O$	1.8	$CuSO_4 \cdot 5H_2O$	4	$CuSO_4 \cdot 5H_2O$	4
		KI	10	KI	10
	µg/100 ml	$FeCl_3 \cdot 6H_2O$	20	$FeCl_3 \cdot 6H_2O$	20
Ca pantothenate	100	$MnSO_4 \cdot H_2O$	40	$MnSO_4 \cdot H_2O$	40
Riboflavin	100	$Na_2MoO_4 \cdot 2H_2O$	20	$Na_2MoO_4 \cdot 2H_2O$	20
Pyridoxamine	100	$ZnSO_4 \cdot 2H_2O$	40	$ZnSO_4 \cdot 2H_2O$	40
Pyridoxal	100				
Pyridoxine	100		µg/100 ml		µg/100 ml
Nicotinic acid	100	Ca pantothenate	40	Ca pantothenate	40
Thiamine	100	Riboflavine	20	Nicotinic acid	40
PABA	10	Pyridoxine	40	Thiamine	40
Biotin	1	Nicotinic acid	40	Inositol	200
Pantetheine	10	Thiamine hydrochloride	40		
Folic acid	1	PABA	20		mg/100 ml
Inositol	200	Biotin	0.2	DL-alanine	19
Lipoic acid	1	Folic acid	0.2	L-arginine-HCl	19
Leucovorin	1	Inositol	200	DL-aspartic acid	60
B_{12}	0.2			L-cystine	2
			mg/100 ml	L-glutamic acid	260
	mg/100 ml	L-histidine	.1	Glycine	2.5
DL-alanine	19	DL-methionine	.2	L-histidine HCl·H_2O	12
L-arginine-HCl	19	DL-tryptophan	.2	L-hydroxyproline	1
DL-aspartic acid	60			DL-isoleucine	48
L-cystine	2			DL-leucine	48
L-glutamic acid	260			L-lysine HCl·H_2O	32
Glycine	2.5			DL-methionine	33
L-histidine HCl·H_2O	12			DL-phenylalanine	39
L-hydroxyproline	1			L-proline	44
DL-isoleucine	48			DL-serine	48
DL-leucine	48			DL-threonine	40
L-lysine HCl·H_2O	32			DL-tryptophan	12
DL-methionine	33			L-tyrosine	33
DL-phenylalanine	39			DL-valine	79
L-proline	44			L-asparagine	20
DL-serine	48			L-glutamine	20
DL-threonine	40				
DL-tryptophan	12				
L-tyrosine	33				
DL-valine	79				
L-asparagine	20				
L-glutamine	20				
Adenine-SO_4	1				
Guanine-HCl	1				
Uracil	1				

* Medium described by Demain and Hendlin (1958).
** Difco.

When the concentration of Proteose Peptone was varied, it was found that the minimal level for growth was about 0.4% and the optimum concentration was 1%. Omission of yeast carbon base gave poor growth. One percent yeast extract (Difco) plus 1% Proteose Peptone stimulated good growth.

The pH of the media used in the initial experiments was not adjusted and ranged from about 4.5 to 6.0. In order to carry out further tests on growth at the most favorable pH, the effect of pH on growth was studied next. The results are shown in FIG. 1. The optimum pH for growth was found to be 4.5. There was little growth at pH 7.0 and no growth occurred at pH 7.5. At pH values below 4.0 growth was

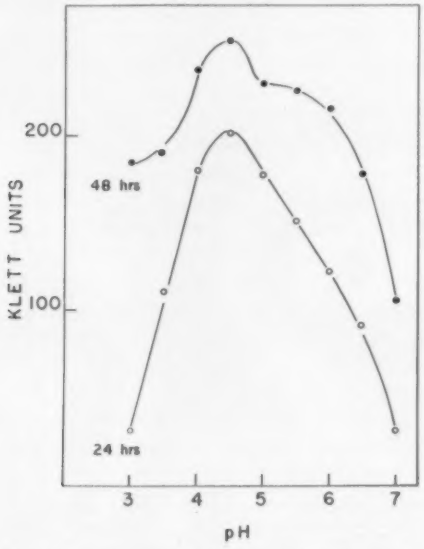

FIG. 1. Relation between growth of *S. guttulata* in yeast autolysate + Proteose Peptone + glucose and the pH of the culture medium. Standing cultures were used.

delayed during the first 24 hours, but after 48 hours this lag had been overcome. In the subsequent experiments the pH of the media was adjusted to 4.5.

Different hydrolysates of casein were tested also. These preparations do not contain nucleic acids or fractions thereof. If they should support growth, it would indicate that the growth factor required by *S. guttulata* is part of a protein molecule. Three commercial preparations were used in the following experiments: an acid and an enzymatic hydrolysate of casein (Nutritional Biochemicals Corp.) and Trypticase (Baltimore Biological Lab.). The last preparation is a pancreatic

digest of casein. The experimental procedures followed were as described above. The casein hydrolysates were dissolved or diluted so that the final concentration was 1%. Yeast carbon base was added to supply minerals and vitamins. Only the medium containing Trypticase supported growth of *S. guttulata*. It is of interest to note that of the two preparations of enzymatically hydrolyzed casein only Trypticase supported growth. Evidently the method or extent of hydrolysis of casein determines whether the preparation satisfies the nutritional requirements of *S. guttulata*.

Experiments were then conducted to find the minimal concentration of Trypticase required in an adequate basal medium. In order to have sufficient nitrogen in the medium, 1% acid-hydrolyzed casein (NBC) was added to the test media to supplement the nitrogen requirement of *S. guttulata*. Exploratory concentrations of Trypticase used in these tests were 0.2, 0.5 and 1.0 % (w/v) in yeast carbon base plus acid-hydrolyzed casein. None of these media supported growth. Thus, 1% Trypticase in yeast carbon base supported growth, but growth was suppressed on addition of 1% casein hydrolysate. The inhibition of growth was also exhibited when an enzymatic hydrolysate of casein (NBC) was used. The inhibition of growth in the presence of 1% of either one of the above hydrolysates could be overcome when the concentration of Trypticase was increased to 2%. Since all three materials are hydrolysates of casein, it would appear from these experiments that the balance of amino acids and peptides in a suitable medium is quite critical.

An attempt was then made to grow *S. guttulata* in certain media employed by previous workers. When malt extract was used it was found to support growth in an erratic fashion. Evidently there are differences between lots of malt extract (Diamalt, Standard Brands, Inc.), since in some trials fair growth was obtained, whereas in others the medium failed or it supported poor growth. This may explain the results of Lodder and Kreger-van Rij (**1952**), who could not subculture *S. guttulata* from their primary enrichment cultures in acidified malt extract. Good growth occurred in a medium in which Parle (**1956**) successfully cultured *S. guttulata*. The stomach extract was prepared by grinding the stomach plus contents with an equal volume of distilled water in a Waring blender. After the preparation was homogenized it was filtered through a Seitz filter. The final medium contained 10% (v/v) of the stomach extract, 1% yeast extract (Difco) and 2% glucose. There was no difference in the rate of growth or in the appearance of cells of *S. guttulata* in this medium as compared to a medium containing autolysate or Proteose Peptone.

Effect of heat on the media. Since some reports in the literature have indicated harmful effects of autoclaving on the growth of certain microorganisms (Rose et al., **1952**; Stanier, **1942**), it was felt desirable to determine if sterilization by filtration would enhance growth over that in autoclaved media. The medium used in these experiments was yeast autolysate prepared in the laboratory (10% v/v), yeast nitrogen base, Difco (0.67%), and glucose (2%). The media were prepared in the following ways: (a) all ingredients were added together and autoclaved, (b) the three ingredients were autoclaved separately in threefold strength, and combined aseptically before use, (c) all ingredients were combined and sterilized by filtration through a Seitz filter. Growth in all media was equally good. Even when autoclaving was done for 30 minutes, growth was not impaired. This confirms the finding by Parle (**1956**) that the growth factor(s) required by *S. guttulata* is heat-stable and no inhibitory products are formed during autoclaving.

Characterization of the growth factor in natural media. It was shown that a fraction in yeast autolysate is required for growth. First, the effect of the time of autolysis (governing the degree of breakdown) on the ability of the resultant autolysate to support growth was studied.

Baker's yeast [1] was allowed to autolyse in the presence of toluene at 55° C for 6, 24, 72 and 120 hours and at room temperature for 120 hours. After autolysis the media were brought to a boil and filtered with the aid of diatomaceous earth. An extract of fresh yeast was prepared by autoclaving equal volumes of yeast and water. Two percent glucose was added to 10% (v/v) of the different autolysates and to the yeast extract and these media were used for growth experiments.

Growth occurred only in media containing digests that had been incubated for 72 or 120 hours at 55°. All other preparations failed to support growth.

On the basis of the above results it can be inferred that the factor(s) is part of a larger molecule or is conjugated with another molecule, and has to be hydrolyzed or released, before it is available to *S. guttulata*.

Next, Proteose Peptone and yeast autolysate were subjected to dialysis to determine whether the growth factor(s) is dialyzable.

One hundred ml of a 2% solution of Proteose Peptone and 100 ml of a 20% (v/v) yeast-autolysate solution were placed in dialysis bags which were immersed in graduate cylinders containing 100 ml of distilled water, and rotated slowly for six hours.

The solutions outside the bags were collected and the dialysis bags

[1] Commercially produced one pound yeast cakes were used. No difference was detected between three available brands.

placed in running water and rotated for an additional six hours. Yeast carbon base was added to the diffusate and to the non-dialyzable fraction of both materials. In addition the two fractions of each material were recombined to serve as a control. All media were found to support growth.

It is evident that a dialyzable component(s) of relatively small molecular weight from Proteose Peptone or from yeast autolysate can substitute for the original materials. The fact that the non-dialyzable fraction still supported growth may be due to the fact that (1) not all of the growth factor(s) is removed during dialysis for 12 hours; in this connection Biserte and Scritan (**1953**) reported that malt extract had to be dialyzed for 6 days to completely remove amino acids and certain peptide fractions; or (2) the growth factor(s) is also present in a non-dialyzable form, but available to the yeast; or (3) both possibilities mentioned above may occur.

Growth in synthetic media. The various data thus far obtained provided some evidence that a dialyzable protein hydrolysate of relatively small molecular weight can serve as growth factor for *S. guttulata.* Since not all protein hydrolysates are suitable, the amino-acid balance appears to be critical.

A medium recently developed by Demain and Hendlin (**1958**) for nutritional studies of a strain of *Bacillus subtilis* was used first. This medium (TABLE I, column 1) contains 21 amino acids and amides, salts, trace elements, vitamins, two purines and a pyrimidine. A 100 ml sample of the above medium was kindly supplied by Dr. Demain. It was adjusted to pH 4.5 and 5 ml fractions were used in test tubes. Good growth occurred after 3 to 4 days, but the rate of growth was slower than that occurring in natural media. In synthetic media an extra one or two days are required to obtain the same growth as that occurring in natural media. Eight consecutive transfers were made in the defined medium. Next, attempts were made to simplify this synthetic medium. First, it was tried to substitute yeast carbon base (Difco) for the minerals and vitamins in the medium of Demain and Hendlin. The composition of yeast carbon base is given in column 2 of TABLE I. Adenine, guanine, uracil, 1% of additional glucose, and the 21 amino acids listed in column 1 of TABLE I were added to yeast carbon base. This medium did not support growth of *S. guttulata.* This might be due to: (1) the lack of certain vitamins, *i.e.,* pantetheine, pyridoxamine, pyridoxal, lipoic acid, Leucovorin or B_{12}, present in the original medium; (2) differences in mineral composition; (3) an imbalance between the components present. The latter case is complex.

It means that one of the components in the modified medium, which was present also in the original medium, could have become inhibitory on removal of one or more of the above 6 vitamins, or because of changes in the mineral composition of the medium. Since not all of these six vitamins were available as pure compounds in our laboratory, the last possibility was studied first. The medium, containing yeast carbon base, was prepared again and certain groups of compounds were omitted. It was found that upon removal of adenine, uracil and guanine from the medium growth took place. Eight consecutive transfers were effected through this medium.

When only one of the purine or pyrimidine compounds was added back to the medium normal growth took place. In the presence of any two of these compounds growth was inhibited to a large extent. The presence of all three inhibited growth completely.

Experiments were then performed to determine which of the nine vitamins present in yeast carbon base (TABLE I, column 2) are essential for growth of *S. guttulata*. Vitamin-free yeast base (Difco) to which 1% of additional glucose and the same twenty-one amino acids were added was used as the basal medium for these experiments. Vitamin-free yeast base (Difco) differs from yeast carbon base (Difco) in that it lacks vitamins. Also it contains 0.5% of $(NH_4)_2SO_4$ and L-histidine, DL-methionine and DL-tryptophane in concentrations of 1 mg, 2 mg and 2 mg/100 ml, respectively. The nine vitamins used in these tests were prepared in a 100 × concentration and mixed in such a manner as to give nine vitamin solutions (10 ×) each devoid of a single vitamin. A solution containing all the vitamins was also prepared. The solutions were sterilized by autoclaving, and 0.5 ml of each of the mixtures was added to an Erlenmeyer containing 4.5 ml of a sterile basal medium. The flasks were inoculated with 0.1 ml from a 48 hour culture grown in the synthetic medium. *S. guttulata* did not grow when nicotinic acid, inositol, pantothenic acid and thiamine were omitted singly from the test medium.

A confirmatory test was carried out in which all vitamins, except nicotinic acid, inositol, pantothenic acid and thiamine, were omitted simultaneously. Good growth ensued in the basal medium to which the four vitamins were added. It is possible that the requirement for other vitamins was masked by the presence in the medium of certain amino acids.

Thus, *S. guttulata* can be grown in a defined synthetic medium containing vitamin-free yeast base (Difco), 2% glucose, twenty-one amino acids, and nicotinic acid, pantothenic acid, thiamin and inositol.

Another strain of *S. guttulata*, originally isolated by Parle (**1956**) in New Zealand, was obtained from Drs. S. H. Hutner and A. Cury. It was found that this isolate behaved identically to our own strains with respect to the various growth media. We were unable, however, to induce sporulation in Parle's strain under conditions which favored sporulation of our own isolates (Shifrine and Phaff, **1958**). In addition, it was noted that the New Zealand strain fermented raffinose as did our own strains, whereas Parle reported this property to be negative.

DISCUSSION

Nearly all yeasts isolated from natural substrates can be grown in relatively simple defined media, containing a carbon source, minerals and an inorganic nitrogen source. Vitamins of the B-complex are also required by many strains. Occasionally instances have been reported in which small amounts of amino acids are indispensable for growth. For example, Schopfer and Guilloud (**1945**) found that L-leucine and D-arginine were needed by *Eremothecium ashbyi*. McVeigh and Bell (**1951**) showed that a strain of *Candida albicans* required methionine, and a strain of *Mycoderma vini* required cystine for growth. Recently a number of yeasts have been isolated which have been called "obligate saprophytes of warm-blooded animals" by van Uden and Carmo Sousa (**1957**). Included in this group are *Torulopsis glabrata, Candida bovina, Candida slooffii, Torulopsis pintolopesii* and *Saccharomycopsis guttulata*. *T. glabrata* is the least exacting yeast. *C. bovina* and *C. slooffii* are somewhat more fastidious. *T. pintolopesii* did not respond to peptone, but produced excellent growth on Sabouraud agar to which 0.5% yeast extract (Difco) or meat extract (Difco) was added (van Uden **1952**). *S. guttulata*, as shown in our study, is probably one of the most exacting yeasts. The fact that *S. guttulata* can be grown in a synthetic medium containing a mixture of 21 amino acids and amides shows that the growth-promoting properties of various complex protein hydrolysates can be replaced by one or more amino acids. Our studies have not been carried far enough to state which amino acids are essential. Since acid-hydrolyzed casein, Bacto Peptone and Difco yeast extract do not support growth, but a fully autolyzed bakers yeast, Proteose Peptone and Trypticase are active in this respect, the amino-acid balance is probably quite critical. This is further supported by the observation that addition of increasing amounts of acid-hydrolyzed casein to Proteose Peptone progressively inhibits growth. As pointed out by Magasanik (**1957**) "many of the growth promoting or growth inhibitory effects of components

of complex media may be due to specific interactions at the cell membrane." It is not known at this time whether peptides can replace necessary amino acids or whether ammonia nitrogen can be used, provided essential amino acids are present in low concentration. Observations by Parle (**1956**) and by the writers, that the non-dialyzable fraction of a complex adjunct retained its growth-promoting properties, indicates that peptides might be active in replacing amino acids as growth factors.

SUMMARY

Saccharomycopsis guttulata was grown in media containing certain complex yeast or protein hydrolysates. Good growth was obtained with Proteose Peptone, Trypticase, fully autolysed bakers yeast, Albimi powdered yeast autolysate, or an extract of rabbit stomach. The growth factors are heat-stable and dialyzable. No growth occurred with Bacto Peptone, Difco yeast extract, or an acid or enzymatic hydrolysate of casein (N.B.C. Corp.). These casein hydrolysates inhibit growth in Proteose Peptone. *S. guttulata* was also grown successfully in a synthetic medium containing glucose, minerals, 21 amino acids (and amides), nicotinic acid, inositol, pantothenic acid and thiamine. When adenine, guanine and uracil were added together to this medium growth was inhibited. The optimum pH for growth is 4.5.

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LITERATURE CITED

1. **Biserte, G., and R. Scriban.** 1953. Beer proteins. Wallerstein Lab. Comm. **16**: 339–362.
2. **Bouthilet, R. J., N. E. Neilson, E. M. Mrak and H. J. Phaff.** 1949. The fermentation of trehalose by yeast and its taxonomic implications. Jour. Gen. Microbiol. **3**: 282–289.
3. **Demain, A. L., and D. Hendlin.** 1958. Growth stimulation of a strain of *Bacillus subtilis* by glycine peptides. Jour. Bact. **75**: 46–50.
4. **Lodder, J., and N. J. W. Kreger-van Rij.** 1952. The yeasts—a taxonomic study. North Holland Publ. Co., Amsterdam. 713 pp.
5. **Magasanik, B.** 1957. Nutrition of bacteria and fungi. Ann. Rev. Microbiol. **11**: 221–252.
6. **McVeigh, I., and E. Bell.** 1951. The amino acid and vitamin requirements of *Candida albicans* Y-475 and *Mycoderma vini* Y-939. Bull. Torrey Bot. Club **78**: 134–144.
7. **Parle, J. N.** 1956. The growth of *Saccharomycopsis guttulata*. Antonie van Leeuwenhoek **22**: 237–242.
8. **Remak, R.** 1845. Diagnostische und pathogenetische Untersuchungen. Berlin.

9. **Rose, C. S., E. P. Feldbaum, R. F. Norris and P. György.** 1952. Toxicity of autoclaved cystine for *Lactobacillus bifidus*. Proc. Soc. Expl. Biol. Med. **81**: 709–712.
10. **Schopfer, W. H., and M. Guilloud.** 1945. La culture d'*Eremothecium ashbyi* en milieu synthétique. Experientia. **1**: 22–23.
11. **Shifrine, M., and H. J. Phaff.** 1958. On the isolation, ecology and taxonomy of *Saccharomycopsis guttulata*. Antonie van Leeuwehoek **24**: 193–209.
12. **Snell, E. E.** 1949. Nutrition of microorganisms. Ann. Rev. Microbiol. **3**: 97–120.
13. **Stanier, R. Y.** 1942. The cytophaga group: A contribution to the biology of Myxobacteria. Bact. Rev. **6**: 143–196.
14. **Uden, N. van.** 1952. Zur Kenntnis von *Torulopsis pintolopesii* sp. nov. Arch. Mikrobiol. **17**: 199–208.
15. ——, **and L. do Carmo Sousa.** 1957. Yeasts from the bovine caecum. Jour. Gen. Microbiol. **16**: 385–395.
16. **Wickerham, L. J.** 1951. Taxonomy of yeasts. U. S. D. A. Techn. Bull. No. 1029. 56 pp.

A COMPARATIVE MORPHOLOGICAL AND PHYSIOLOGICAL STUDY OF THE SPECIES OF DIPODASCUS [1, 2]

LEKH R. BATRA [3]

(WITH 21 FIGURES)

SUMMARY

Dipodascus belongs to the family Dipodascaceae (Endomycetales, Hemiascomycetidae). The genus is characterized by lacking ascogenous hyphae and ascocarps, and possessing typically ∞-spored and bifurcate asci. There are three species: *D. albidus* Lagerheim, the type species, *D. uninucleatus* Biggs, and *D. aggregatus* Francke-Grosmann. The distinguishing characters of these species are outlined in a key to the species.

The life history of *D. aggregatus* is described for the first time and is compared with that of the other two species.

PREVIOUS INVESTIGATIONS OF DIPODASCUS

THE GENUS DIPODASCUS AND THE DESCRIBED SPECIES

Lagerheim (**1892**) described *Dipodascus* as a monotypic genus based upon *D. albidus* Lagerheim, reported from the slime flux of *Puya* sp. (Bromeliaceae) in Ecuador. He gave a comprehensive account of its general morphology and life history. He observed the mycelium with monopodial branches arising near the septa. Asci in *D. albidus* were reported to develop in advance of conidia and gemmae and without exception as a result of the fusion of two gametangia.[4] One of the gametangia (female) enlarges and becomes a long, multispored ascus tapering toward the apex. Ascospores were reported to be discharged one by one, forming a ball at the apex of the ascus. No work was done on the cytological aspects of *D. albidus* by Lagerheim.

[1] A portion of a thesis presented to the faculty of the Graduate School of Cornell University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2] The author is grateful to Drs. R. P. Korf, C. H. Uhl, and C. W. Boothroyd for their guidance in this work. Thanks are also due to Dr. Peter Hunt of Birkbeck College, London, for guidance in some experiments on nutrition and physiology of the organisms reported. The writer expresses his appreciation to the individuals who lent dried material or supplied cultures mentioned in this paper.

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[4] Lagerheim used the term "gamete" to designate the gametangium.

Juel (**1902**) isolated *D. albidus* from the slime flux of a species of *Betula* (Betulaceae) in Sweden. He studied (Juel **1902**, **1921**) the cytology and gave further details of the life history of the fungus. While there are 10–12 nuclei in each young gametangium, there are many more in the vegetative cells. Sexual nuclei in the gametangia are indistinguishable from the nuclei in vegetative cells (Juel **1902**). He observed a fusion nucleus in the female gametangium [5] and considered it as an indication of fusion of two nuclei, one from each gametangium.

Dangeard (**1907**) studied *D. albidus* more critically than the previous workers. He was the first to observe the eccentric location of the nucleus in the ascospores, the multinucleate nature of the meristem-arthrospores, fusion of two gametangial nuclei, and the mitotic figure in the third division of the fusion nucleus. Unlike Lagerheim (**1892**) and Juel (**1902**), he found that male and female gametangia were differentiated from the beginning. The female gametangium appears earlier than the male and when they fuse, the former comes to lie at the apex of the latter.

D. albidus was again collected in August 1935 in Colombia by Martin (**1937**), but it could not be cultured.

Kobayasi (**1950**, **1953**) isolated *D. albidus* in Japan and cultured it on liquid and solid media. He obtained asci on malt-extract agar. He also studied fermentation of fructose, glucose, galactose, maltose, saccharose, and lactose by this organism. He confirmed some of the observations made by previous authors on the general morphology of the fungus, but no cytological work was done.

Another species of *Dipodascus*, *D. uninucleatus* Biggs (**1937**), was isolated from a dead pupa of *Drosophila melanogaster* Meigen in Canada. The general morphology and the life history were worked out by the original author. Biggs used several liquid and solid media for culturing *D. uninucleatus*. She failed, however, to give the composition of the liquid media. She found that 2 per cent malt-extract agar gave the best growth.

Dipodascus uninucleatus differs from *D. albidus* in having a yeast-like habit and uninucleate vegetative and reproductive cells, and in lacking meristem-arthrospores.

Dulaney and Grutter (**1950**) cultured *D. uninucleatus* on chemically defined media and showed the organism to be heterotrophic for biotin and vitamin B_1.

The third species, *D. aggregatus* Francke-Grosmann (**1952**), was isolated from the larval tunnels of *Ips acuminatus* Gyll. infesting *Pinus sylvestris* L. in Sweden. No study of its life cycle was made but a

[5] Juel used the term "carpogone" to designate the female gametangium.

brief and somewhat inaccurate morphological description was provided.

Dr. L. J. Wickerham (*personal communication*) isolated a species of *Dipodascus* from gray-white frass taken from the galleries of "Brown Bark Beetles" infesting *Picea engelmanni* Parry in the Medicine Bow Mountains of Wyoming in August, 1950. This was later found by the writer to be *D. aggregatus.*

Korf (**1957**), unaware of Francke-Grosmann's work, described an isolate of *Dipodascus* from the roots of *Pinus resinosa* Ait. collected from Horseheads, N. Y., as *Dipodascus albidus* forma *minor* Korf. This also proved to be *D. aggregatus.*

TAXONOMIC POSITION OF THE GENUS DIPODASCUS

The genus *Dipodascus,* lacking both ascogenous hyphae and ascocarps, and with typically multispored and bifurcate asci, has been treated by some authors as the type of a monotypic family, Dipodascaceae (Dangeard **1907**, Gäumann & Dodge **1928**, Gäumann **1952**). Other authors have treated the genus as allied to *Ascoidea* Brefeld & Lindau and included only these two genera in the family Ascoideaceae (Lagerheim **1892**, Schröter **1897**, Juel **1902**, Bessey **1935, 1952**, Wolf & Wolf **1947**, Alexopoulos **1952**). Lindau (**1900**) also added two doubtful genera, *Oscarbrefeldia* Holtermann and *Conidiascus* Holtermann, to the family Ascoideaceae. Gwynne-Vaughn & Barnes (**1927**) referred this genus to the family Endomycetaceae.

Moreau (**1954**) places both *Ascoidea* and *Dipodascus* in the same family, but uses the family name *Dipodascaceae* instead of the older name *Ascoideaceae.*

The genus has been variously assigned to the orders Plectascales (Gwynne-Vaughn & Barnes **1927**), Endomycetales (Gäumann **1926**, Gäumann & Dodge **1928**, Wolf & Wolf **1947**, Alexopoulos **1952**, Gäumann **1952**, Martin **1954**), Saccharomycetales (Bessey **1935, 1950**), and Dipodascales (Moreau **1954**).

The members of the genus are usually included in the class Ascomycetes, subclass Hemiascomycetidae (Lagerheim **1892**, Varitchak **1928, 1931**, Nannfeldt **1932**, all as "Hemiasci"; Schröter **1897** as subclass "Hemiascineae"). Gäumann (**1926**), and Gäumann & Dodge (**1928**), term this subclass as "Hemiascomycetes" and Gäumann (**1952**) as "Protascomycetes."

Moreau in a complete revision of the fungus classification includes the order Dipodascales along with other orders, Protoascales, Protomycetales, Ashbyales, Spermophthorales and Synascales in the class Periascomycetes, which is not subdivided into subclasses.

On the basis of the present work, the writer includes only two

genera, *Dipodascus* and *Helicogonium* White (**1942**), in the family Dipodascaceae. The genus *Helicogonium* is characterized by the possession of bifurcate asci formed as a result of the fusion of two gametangia arising from different branches. The asci are 8-spored and become ∞-spored on maturity by budding of the originally 2-celled spores. The asci do not stain with iodine solution. No asexual reproductive bodies have been observed. *Helicogonium* appears closely related to *Dipodascus* and is referred to this family for the first time. He excludes *Ascoidea*, treating it as the type genus of the family Ascoideaceae. He is further of the opinion that the family Dipodascaceae belongs to the order Endomycetales. If future work on Dipodascaceae shows that its members differ from other Endomycetales, it could be referred to the order Dipodascales erected by Moreau (**1954**). A Latin diagnosis will have to be provided to validate the order.

MATERIALS AND METHODS

1. *Cultures utilized.* The following isolates were used throughout the work:

Organism	Source
Dipodascus aggregatus Francke-Grosmann	Dr. Helene Francke-Grosmann from the galleries of *Ips acuminatus* Gyll. attacking *Pinus sylvestris* L., collected in Sweden in 1950. Francke-Grosmann (**1952**).
D. aggregatus Francke-Grosmann	Writer's isolates from frass of *Ips pini* Say and an earthworm, collected from Horseheads, N. Y. in 1957.
D. aggregatus Francke-Grosmann (an isolate described by Korf (**1957**) as *D. albidus* forma *minor* Korf).	Dr. R. P. Korf from the roots of *Pinus resinosa* Ait. from Horseheads, N. Y., collected in 1956. Korf (**1957**).
D. aggregatus Francke-Grosmann (an isolate sent to the writer as a *Dipodascus* sp.)	Dr. L. J. Wickerham from the frass of "Brown Bark-beetles" attacking *Picea engelmanni* Parry in Medicine Bow Mountains of Wyoming, U.S.A., collected in 1950.
D. uninucleatus Biggs	Dr. Rosemary Biggs from a dead pupa of *Drosophila melanogaster* Meigen in Canada. Biggs (**1937**).
D. uninucleatus	From Carolina Biological Supply Company, received in 1957.
D. uninucleatus	Dr. H. J. Phaff from a *Drosophila* caught in a vinegar factory in Berkeley, California. Isolated in 1950.
D. albidus Lagerheim (non-ascigerous when studied)	Dr. Y. Kobayasi from exudates of trees in Nippara, Japan. Collected in 1950. Kobayasi (**1952**).

2. *Media and cultural conditions.* Stocks of these isolates were maintained on malt-extract agar at 5° C and were subcultured on fresh medium every 6–10 weeks. When using solid media, the isolates were grown in acid-cleaned 9 cm Petri plates or in Pyrex test tubes. When liquid media were employed, 50 ml of the medium was placed in 125 ml Pyrex Erlenmeyer flasks. All cultures were grown at room temperature (26 ± 1.5° C) unless indicated otherwise.

In morphological studies, corn-meal agar was selected for routine work. This gave sexual stages which could be easily examined, as fewer meristem-arthrospores[6] (Hughes **1953**) were produced in *Dipodascus aggregatus.*

3. *Isolation methods.*

a. *Isolation of Dipodascus aggregatus from nature.* The organism was isolated from a dead earthworm, collected from the floor of a mixed forest, from the frass of *Ips pini,* and from soil. The writer made several unsuccessful attempts to isolate the fungus from the inside of roots of both dead and live stands of *Pinus resinosa* and *P. strobus* L.

In the middle of the body of a dead earthworm was observed a shiny, yeast-like, dry mass of mycelium, which became gelatinous in water. This contained myriads of meristem-arthrospores, suggestive of *Dipodascus aggregatus.* The few middle segments of the earthworm harboring this gelatinous mass were passed through a series of flasks containing yeast-extract broth, followed by several flasks of sterilized distilled water. The segments of the body were then plated on agar. After 4–6 hours, several drops of the meristem-arthrospore suspensions in yeast extract and in distilled water were drawn from these flasks and dilution plates were made with acidified malt extract, following Koch (**1883**) as modified by McNew (**1938**).

A few days later, along with other bacterial and fungal contaminants, *D. aggregatus* was isolated from several dilution plates of material from the body of the earthworm.

Frass taken from the galleries of *Ips pini* infesting dead trees of *Pinus resinosa* growing in the vicinity of Horseheads, New York, and soil samples from the same area, were suspended in sterile distilled water. These suspensions were centrifuged to remove the larger frass or soil particles respectively. The supernatant was either streaked or plated by the soil-dilution method as described by Riker and Riker (**1936**). Typical colonies of *D. aggregatus* were obtained from such plates within a week.

[6] Meristem-arthrospores are conidia formed in a basipetal succession by the fragmentation of terminally meristematic conidiophores.

b. *Single-spore isolations.* McNew's (**1938**) poured-plate technique for the isolation of single ascospores was tried but could not be relied upon because of the sticky nature of the material.

The technique of W. J. Martin (**1943**) for isolating fungus spores from slides exposed to air was modified to suit the requirements. A sterilized coverglass was gently placed on the edge of the colony of *D. aggregatus.* The ascospore balls stuck to the underside of the coverglass, which was then lifted up with a pair of forceps and dropped into a flask of sterilized distilled water. The spore suspension was thoroughly shaken and streaked aseptically on thin layers of potato-dextrose agar in Petri plates.

Within 24 hours at room temperature, many ascospores produce germ tubes. The germinating ascospores were then picked up under a binocular microscope with a transfer needle and transferred to malt-extract-agar slants. Ascospores were also picked up with a micromanipulator. This was especially important in *D. uninucleatus,* where the ascospores are very small.

4. *Methods for microscopic examination of living material.* Slide cultures and hanging-drop cultures were used to observe development of various morphological entities. Cultures grown on cellophane squares (Carmichael **1956**) proved very useful in studying the formation, development, and fusion of gametangia of *Dipodascus* spp. A 25 mm square of sterilized cellophane was placed on malt-extract agar and seeded with spore suspension. After the organism had grown and reached a particular stage, the cellophane square was lifted up with a pair of forceps. It was either examined directly under water or processed by fixation and staining for permanent mounts. Development of gametangia was followed in living material. Three-day-old cultures grown on thin layers of Difco potato-dextrose agar in 9 cm Petri plates were placed on the stage of a light microscope and the lids were removed. After a particular stage had been located, the bottom of the Petri plate was fastened to the stage with adhesive tape. Drying of the material under examination during the periods between observations was prevented by placing a plastic hood over the microscope and introducing a damp cloth into the chamber thus formed. The earliest possible stages in the development of a gametangium were periodically examined under a magnification of 500 ×.

5. *Fixation and staining procedures.* The material on a coverglass, microscopic slide, or cellophane square was fixed in a dish containing FAA (Formalin, 5 ml; acetic acid, 5 ml; and 50 per cent ethyl alcohol, 90 ml). Likewise, aerial mycelium with desired sexual stages was

gently scraped off the agar in Petri-plate cultures with a razor blade and fixed for 1–2 hours. All material was then washed twice with 50 per cent ethyl alcohol. It could be stained immediately, or stored in the alcohol. For temporary mounts, the material was stained in 0.5 per cent aqueous phloxine and mounted in glycerine. For permanent preparations, coverglass cultures and mycelium scraped from the surface of agar plates were stained by Heidenhain's iron-haematoxylin technique as given by Johansen (**1940**), and by the technique of Hoffmeister (**1953**) with trypan blue.

Much better results were obtained in staining the nuclei by the technique of Dr. Deana Klein (unpublished).[7] Mycelium on cellophane squares was fixed in Carnoy's solution (3:1 of 100 per cent ethyl alcohol and glacial acetic acid respectively), taken down to water and hydrolyzed in 1 N HCl for 5 minutes at 60°. The material was washed in two changes of cold 1 N HCl and stained for 30 minutes in freshly prepared Azure A stain, consisting of one part of 2 per cent aqueous Azure A and one part of a mixture of equal proportions of 1 N HCl and 10 per cent aqueous sodium bisulphite. The material was then counterstained for a few seconds in a 0.5 per cent solution of eosine Y in 95 per cent ethyl alcohol. After dehydration, the cellophane square, with the material still attached to it, was mounted in a thin layer of neutral Canada balsam.

The measurements of asci, ascospores, and oidia were taken in distilled water and the range given is based on 100 such observations. Dried material was first revived in 2 per cent aqueous KOH solution and then immersed in distilled water.

6. *Methods for study of the nutritional requirements and some physiological aspects of Dipodascus spp.*

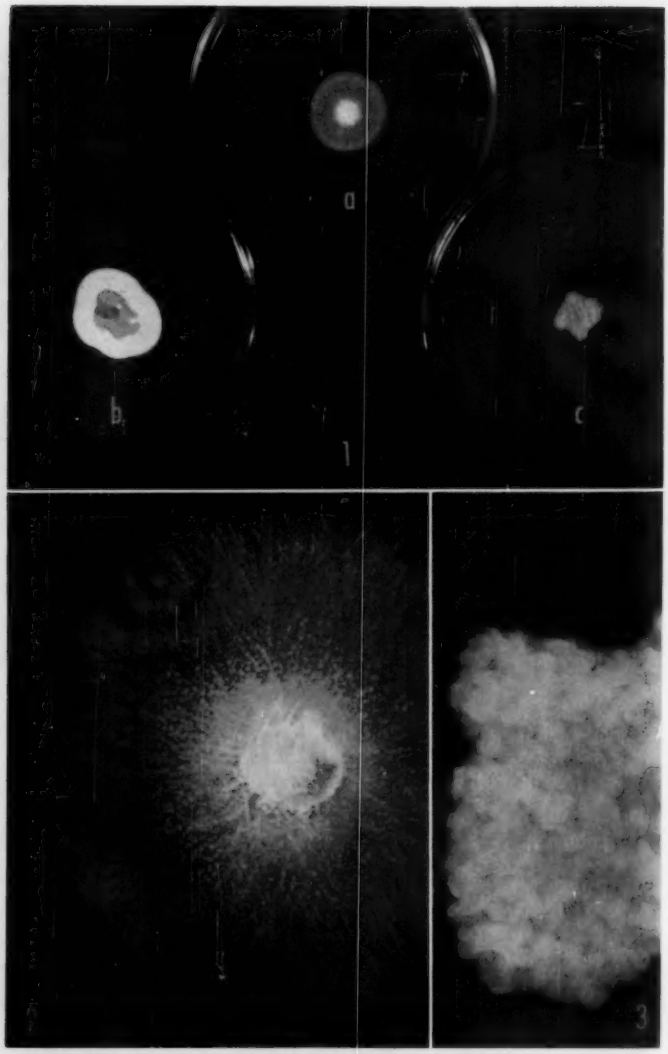
a. *The basal medium.* A modified Pfeffer's medium (Pfeffer **1897**) was used as the basal medium in thoroughly cleaned 125 ml Erlenmeyer flasks: NH_4NO_3,[8] 3 g; KH_2PO_4, 2 g; $MgSO_4 \cdot 7H_2O$, 0.75 g; $FeSO_4$, 0.002 g; Dextrose,[9] 10 g; distilled water, 1 l. A vitamin mixture[10] adopted from Dulaney and Grutter (**1950**) was added to yield the following final concentration (in mg/l): ascorbic acid, 0.10; biotin, 0.005; inositol, 5.00; pyridoxine hydrochloride, 0.10; riboflavin, 0.10; and thiamine (aneurine) hydrochloride, 0.10. Wherever vitamins were used,

[7] Verbal communication to the writer by Dr. Lindsay S. Olive, Columbia University.

[8] Except in the case of nitrogen utilization experiments.

[9] Except in the case of carbon utilization.

[10] Except in the case of vitamin requirement experiments.



FIGS. 1-3.

FIG. 1. Fifteen-day-old colonies of three species of *Dipodascus* on malt-extract agar, somewhat reduced. a, *D. aggregatus* (CUP 44260); b, *D. albidus* ("N.I.

they were sterilized by filtration through a Seitz filter and added to the previously autoclaved medium.

All experiments were performed at least twice and in each case, three replicates of each treatment were set up. Testing for fermentation and acid production was performed in Einhorn tubes, following the method of Stelling-Dekker (**1931**).

b. *Preparation of "inoculum."* (Suspension of ascospores, arthrospores, or both, or yeast-like cells.) In all cases sub-isolates of monosporic cultures were used. Cultures grown in glucose-yeast-extract broth (1 per cent glucose, 0.8 per cent yeast extract by weight) in 125 ml Erlenmeyer flasks were washed twice with distilled H_2O and used to plant a few flasks containing basal medium. Forty-eight hours later, the ascospores, meristem-arthrospores, or sprout cells, as the case might be, were withdrawn from this medium, using a pipette whose capillary end was wrapped in two layers of cheesecloth. This procedure prevents the gelatinous mycelium from entering into the suspension. This suspension was diluted with sterile distilled water to give 300–450 spores/mm^2 when mounted in a thin film of water. Each flask or Petri plate was seeded with one-tenth ml of this suspension. In experiments to test the effects of vitamins, the undiluted spore suspension was pipetted into centrifuge tubes and washed twice with sterile distilled water. This was then diluted and used as mentioned above. Cultures in liquid media were harvested on the twenty-first day after inoculation. The growth was measured by weighing the dry mycelium.[11]

7. Deposit of material and abbreviations. Material pertaining to this work has been deposited in the Cornell University Plant Pathology Herbarium with accession numbers 43905, 44260, and 44778.

The cultures of *Dipodascus aggregatus* Francke-Grosmann from New York State have been deposited with Centraal-bureau voor Schimmelcultures, Baarn, Netherlands, with the American Type Culture Collection, Washington, and with the ARS Culture Collection, Investigations Fermentation Laboratory, Peoria, Illinois. The abbreviations of the herbaria are taken from those proposed by Lanjouw and Stafleu (**1956**). Colors given in quotation marks are from Ridgway (**1912**).

[11] Data pertaining to such observations is omitted here and may be consulted in the above-mentioned thesis.

2087"); c, *D. uninucleatus* (*CBS*). FIG. 2. Enlargement of FIG. 1a, showing needle-shaped synnemata in *D. aggregatus*, × 8. FIG. 3. Enlargement of FIG. 1c, showing yeast-like nature of a colony of *D. uninucleatus*, × 8.

OBSERVATIONS

1. *Mycelium.* In pure cultures, abundant mycelium is produced by all isolates studied (FIGS. 1a, 2). The growth in general is slow. Vigorously growing colonies of all isolates are "Pinkish Vinaceous" to "Light Purplish Vinaceous" in color. The mycelium is septate; its cells are exclusively uninucleate and with age become vacuolated. It is branched, the branches being always developed adjacent to the septa. Some of the branches grow into the culture media; others are aerial. Reserve food particles of various sizes and shapes are scattered in the cytoplasm throughout the cells of the mycelium. These particles contrast sharply with the rest of the cytoplasm under the phase microscope. They are, however, distinguishable from the nuclei because of their greater refractivity (FIG. 18).

The nucleus may lie anywhere in the cell but most often is pressed against the wall midway of the long axis of the cell. It has rarely been seen near a septum. The mycelial nuclei in stained preparations measure from 1.5–2.5 μ.

2. *Asexual reproductive structures.*

a. *Meristem-arthrospores. D. aggregatus* produces meristem-arthrospores, referred to as "oidia" by Francke-Grosmann (**1952**). They are borne on hyphae of indeterminate growth which are indistinguishable from the rest of the vegetative branches. The meristem-arthrospores develop basipetally by the successive division of the nucleus in the meristematic cell. Thus the younger arthrospores imperceptibly grade into the meristematic cells, while the older ones are distinct and lie towards the apex (FIGS. 4, 16a).

As the new meristem-arthrospores are formed, the older and mature ones are pushed aside, sometimes making a zigzag chain of cells. When the nutrients in the medium are suboptimum, meristem-arthrospores usually appear within 24 hours from plating. Sometimes they form gelatinous masses reaching a diameter of 0.5–1 mm, giving a warty appearance to the colony. This was observed on alkaline media in particular.

The meristem-arthrospores are uninucleate, with the nucleus almost always lying in the center of the cell. On either side of the nucleus there may be one or two reserve food particles which, if not stained properly, may be mistaken for nuclei (FIG. 5). Meristem-arthrospores germinate in a drop of yeast-extract broth within 24 hours, giving a typical mycelium bearing asexual and sexual reproductive structures.

b. *Synnemata.* In older cultures, needle-shaped synnemata develop in abundance, giving a woolly appearance to the colony. They are usually 1–2 mm long (rarely 4 mm long) and from 0.5 to 1 mm broad at the base (FIG. 2).

Each synnema is a fasciculate aggregation of gelatinous hyphae, indistinguishable from the rest of the mycelium, and bears meristem-arthrospores. The tips of the synnemata are usually curled like a whiplash.

During pH studies with *D. aggregatus* and *D. albidus,* it was observed that synnemata are regularly formed below pH 5 (in both fungi). Whether this is a direct or indirect effect could not be determined.

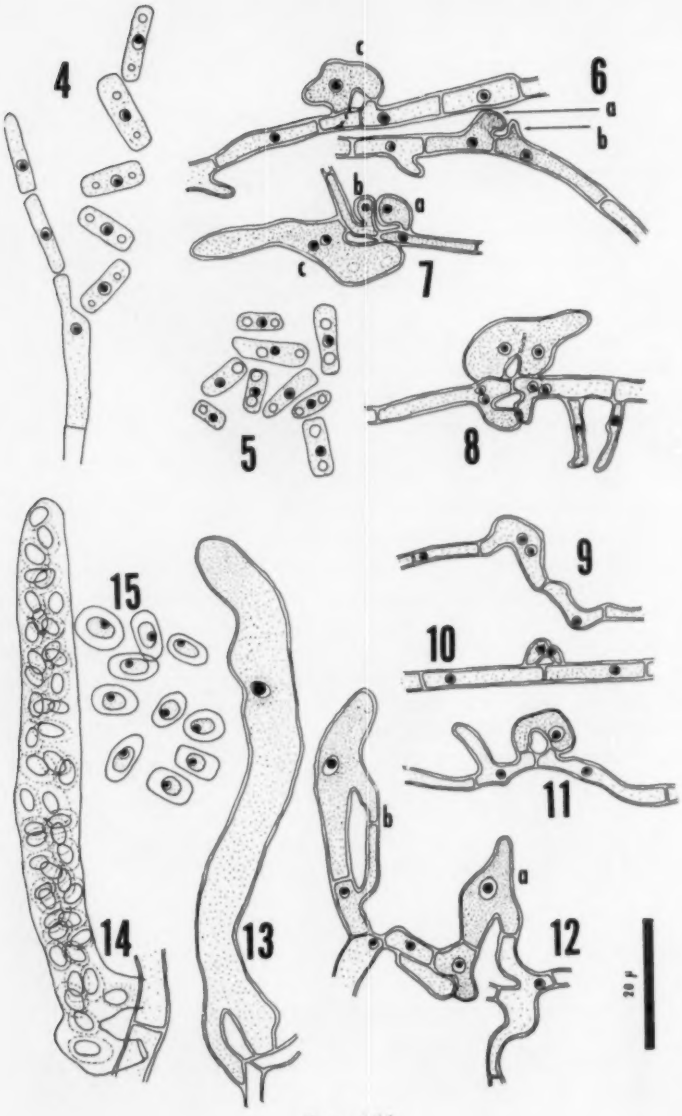
3. *Sexual reproductive structures.* *D. aggregatus* is homothallic, as are the other two species, *D. albidus* and *D. uninucleatus.* Mycelium obtained from either a uninucleate ascospore or a uninucleate meristem-arthrospore bears gametangia and subsequently forms asci. Stages in the development of gametangia from their initiation, the subsequent fusion resulting in the formation of asci, and the liberation of ascospores were closely followed under the phase-contrast microscope. These observations were corroborated by study of fixed and stained material, and by study of living material under a light microscope.

a. *Gametangiogenic cells.* At first the hyphae bearing gametangia are indistinguishable from vegetative hyphae or hyphae bearing meristem-arthrospores. Asexual and sexual reproductive structures may be borne on the same hypha. The cells which take part in sexual reproduction are almost always intercalary and in pairs. These cells are smaller and their contents stain more deeply than the adjacent cells. They are uninucleate, with their nuclei slightly larger than those of vegetative cells, and give rise to gametangia. The writer terms these as *gametangiogenic cells* (FIGS. 6a & b, 16b).

Ordinarily a gametangiogenic cell retains its uninucleate condition. In some cases, however, the nucleus from such cells may itself move into a gametangial initial without undergoing a division. This results in the occasional absence of a nucleus from such gametangiogenic cells (FIGS. 6c, 7b). The hyphae in the sexually fertile region of the mycelium frequently intertwine in vigorously growing cultures.

b. *Gametangia.* *Dipodascus aggregatus* is anisogamous. The two gametangia are of different shape and size from their initiation. They are uninucleate throughout (FIGS. 6a & b, 7a & b).

The gametangia develop in 2–3-day-old cultures on any of the solid media tried. They are also formed in liquid media containing soluble starch and ammonium as carbon and nitrogen sources, respectively. On



FIGS. 4–15.

liquid media, their formation is usually delayed and they are formed only at the surface of the medium.

At first, vegetative hyphal branches and gametangial initials appear similar. Both are initiated usually near a septum. After some time, those initials that are destined to become gametangia are robust, slow growing, and with denser cytoplasmic contents (FIGS. 6a & b). Vegetative branches are narrower, with vacuolated cytoplasm and are fast growing.

A single *gametangial initial* arises from the gametangiogenic cell as a swollen protuberance, usually a few cells behind the hyphal apex. The writer terms this the *female gametangial initial*. The cytoplasmic contents of this protuberance stain deeply throughout (FIG. 6a).

The single nucleus in the gametangiogenic cell bearing a female gametangial initial divides, and one daughter nucleus migrates into the gametangial initial, the other being left in the gametangiogenic cell. The female gametangial initial is cut off by a septum close to its point of origin from the gametangiogenic cell, thus forming the female gametangium (FIG. 7a).

A few hours after the formation of the female gametangial initial, another hypha-like protuberance is formed, usually from the contiguous gametangiogenic cell. The writer terms this the *male gametangial initial*, which is always smaller and narrower than the female gametangial initial (FIG. 6b). As in the development of the female gametangium, the nucleus in this gametangiogenic cell also divides, and one nucleus migrates into the male gametangial initial, the other being left in the gametangiogenic cell. With the formation of a septum between the two, the male gametangium is delimited from the gametangiogenic cell (FIG. 7b).

FIGS. 4–15. *D. aggregatus*, drawn with the aid of a camera lucida at a magnification of 3000 × and reduced here to 1000 × in reproduction (FIGS. 4–13 stained in iron haematoxylin, FIGS. 14, 15 in acid fuchsin). FIG. 4. Formation of meristem-arthrospores. FIG. 5. Mature meristem-arthrospores. FIG. 6. a, Female gametangial initial arising from a gametangiogenic cell. b, Male gametangial initial arising from a gametangiogenic cell. c, Two fused gametangia and the resultant fusion nucleus; but note that there is no nucleus in the gametangiogenic cell giving rise to female gametangium. FIG. 7. a, Mature female gametangium. b, Mature male gametangium. c, Young ascus prior to nuclear fusion. FIG. 8. The wall between the two gametangia has partially dissolved, and the nuclei in the two gametangiogenic cells have again divided. FIG. 9. Fusion of two terminal gametangia. FIG. 10. Male and female gametangia fusing, on poor nutrient supply. FIG. 11. Two fused gametangia with a fusion nucleus, on poor nutrient supply. FIG. 12. a and b, Two young asci arising from pseudostalked gametangia. FIG. 13. Young ascus with a fusion nucleus, on rich nutrient supply. FIG. 14. Mature ascus with 48 ascospores. FIG. 15. Mature capsulate ascospores.

In rare cases the writer has observed a nucleus dividing in a gametangial initial. In such cases the gametangiogenic cell is enucleate. Presumably one of the resultant daughter nuclei migrates back into the gametangiogenic cell, and one remains as the gametangial nucleus.

Neither of the gametangial initials is delimited by a septum from its gametangiogenic cell until they come in contact with each other. Delimitation of the female gametangium always precedes that of the male gametangium (FIGS. 7a & b).

Sometimes the septum separating the gametangiogenic cell and the fusing gametangium is formed higher up in the base of the latter. This is common in those cases where the fusing gametangia arise from different hyphae. Such cases may give a false impression, the fusing gametangia apparently being supported on stalks (FIGS. 12a & b).

In most cases, the gametangia and the developing asci initially lie more or less at right angles to the gametangiogenic cells. The mature asci, however, may be oriented in an irregular fashion because of crowding.

Successive formation of more than one gametangium from a gametangiogenic cell is the rule rather than the exception in *D. aggregatus*. Any time after the fusion of the two gametangia, new gametangia may be initiated from either, or most frequently from both of the gametangiogenic cells. It was observed that a gametangiogenic cell supporting the female gametangium gives rise only to other female gametangia, and similarly a gametangiogenic cell bearing male gametangia gives rise only to male gametangia (FIGS. 8, 16c). One to four gametangia may develop from each gametangiogenic cell. The second and subsequent gametangia thus formed are usually smaller. The asci developing from such gametangia are also smaller and with fewer ascospores.

After the apices of the gametangia have fused and the intervening walls have dissolved away, the contents of the male gametangium migrate into the female gametangium (FIGS. 8, 10). This process, from the first appearance of the female gametangial initial to the migration of the protoplasmic contents from the male gametangium, usually takes from 18 to 36 hours.

In haematoxylin-stained preparations it was observed that each gametangial nucleus comes to lie near the apex, very close to the walls between the fusing gametangia (FIGS. 7a & b). The intervening walls dissolve away and the male gametangium, after having emptied its contents into the female gametangium, appears to be an appendage attached to the latter. The two nuclei previously situated at the junction of the

fusing gametangia move into the center of the female gametangium (FIGS. 7c, 9).

c. *Asci.* Following plasmogamy, the female gametangium rapidly enlarges. The dicaryotic phase is of very short duration and ascogenous hyphae are absent. Fusion of the two nuclei takes place in the middle of the enlarging female gametangium which may now be termed the young *ascus*. The fusion nucleus attains a size of 3–4 μ (FIGS. 7c, 11, 12a & b, 13).

The diploid nucleus does not undergo a resting period and after a few hours divides repeatedly. It was not possible to stain clearly the nuclei after the 8-nucleate stage. The protoplasm in the young ascus appears very refractive and is sljghtly denser towards the apex. Six to eight hours after plasmogamy it was noted in several cases, using material stained with Hoffmeister's Trypan blue, that the fusion nucleus had already divided 2–3 times. Twenty-four hours after gametangial fusion, the segments of the cytoplasm cut out around the ascospores are clearly visible. Nuclei can be seen as small rounded bodies lying eccentrically in the ascospores.

Presumably meiosis takes place in the ascus and the subsequently formed uninucleate ascospores give rise to haploid thalli bearing gametangia. The second division of the fusion nucleus in the ascus has been observed and the gametic number of chromosomes is suspected to be 4 or 5.

The epiplasm left over after the delimitation of ascospores forms a gelatinous sheath around the spores. In mature ascospores, this sheath appears to be very conspicuous and, in spores still within the ascus, forms an angular capsule. Where such capsules of two adjacent ascospores overlap, a dark but sharply distinct lunar area is observed. This area soon disappears when the ascospores are mounted in water (FIG. 17).

A typical ascus of *D. aggregatus* is cylindrical. It is bifurcate at the base and rounded towards the apex. The size of the two supporting prongs (the old gametangia) and the ascus and the number of ascospores per ascus are dependent upon the nutrients in the medium. The number of ascospores may be 8, 12, 16, 24, 32, 48 or 64 with 24-spored and 32-spored asci more common (FIGS. 14, 17, 19, 20, 21). Asci with such odd numbers as 13, 17, 19 and 33 have also been encountered. Generally, the asci formed in the first week after planting are larger and broader than those formed later. Also, as the colony grows older, fewer-spored asci are commoner than those with 24 or 32 spores. The

ascospores in the ascus are uniformly distributed and tightly packed and there is no aggregation near the apex.

The ascus wall is of uniform thickness throughout. At the tip of the ascus an irregular pore is formed, presumably by the dissolution of the wall in this area. The asci do not stain in iodine solution.

Frequently in cultures growing on poor nutrients or in coverglass cultures the prongs of the ascus are hardly visible. This gives an impression at first glance that the asci are not bifurcate. Such asci on staining with a warm solution of 1 per cent cotton blue in lactophenol and mounting in 50 per cent lactic acid show up their bifurcate nature clearly. Presumably the two gametangial initials did not grow appreciably before they fused, resulting in short prongs.

Occasionally the hyphae have been seen to bear asci terminally. Asci thus formed are also a result of fusion of two gametangia. In some cases the writer has observed that the penultimate cell is devoid of any nucleus. In several cases it has been observed that a small gametangium arising from the penultimate cell may fuse with the terminal cell and give an appearance of a clamp connection or crozier. It is possible that in those cases where the penultimate cell is empty, the nucleus might have migrated through the septum between the two cells, and, after the fusion of the two nuclei, given rise to a terminal ascus. But it is yet to be learned whether the size of the fusion nucleus is as large as that of those in normal asci reported here, or whether, as Biggs (**1937**) suggested for *D. uninucleatus,* such asci arise parthenogenetically.

Intercalary asci without any bifurcation have also been observed. Either 1) the intercalary septum breaks down, the two nuclei fuse and the ascus is formed, or 2) there is single nucleus in a protuberance which becomes a parthenogenetic ascus.

d. *Ascospores.* The ascospores in *D. aggregatus* are viscid. They are hyaline to subhyaline, broadly ellipsoid, or somewhat polygonal when packed in a mature ascus. They are surrounded by a thick, hyaline, mucilaginous sheath of epiplasm in the ascus, are smooth-walled, non-amyloid and arranged in a uniseriate fashion. Sometimes portions of the ascus, or even whole asci, may have biseriately arranged ascospores. The ascospores measure (2.5)3.5–5.5 × (1.7)3–3.5 μ without the mucilaginous sheath and 5–8 × 5–6 μ with it (FIGS. 15, 20, 21).

The mature ascus appears to be greatly distended from the swelling of the mucilaginous sheaths of the ascospores. This gives an irregular shape to the ascospore capsule. The ascospores are discharged one by one through the narrow pore of the ascus formed at the tip. The dis-

charged ascospores form a sticky ball which remains attached to the tip of the ascus for a long time (FIG. 17).

The ascospores have no resting period and germinate within 24–36 hours in yeast broth or in tap water at room temperature. The gelatinous capsule that surrounds the ascospores swells greatly and finally becomes so hyaline that it is not possible to distinguish it from the ascospore wall under an ordinary light microscope. At this stage the ascospores appear subglobose and greatly distended, varying in measurements, 8–10 × 7–8.5 μ. Usually only one germ tube is given out from the germinating ascospore but two germ tubes, one from either pole, are also not uncommon.

Under certain conditions, especially in liquid cultures, the ascospores may germinate in dehisced asci. When a germ tube is given out, most of the cytoplasm along with the nucleus migrates into the tip and a large central vacuole appears in the ascospore. With a favorable supply of nutrients, the germ tube grows and branches profusely, but in tap water it may start forming typical meristem-arthrospores before it branches.

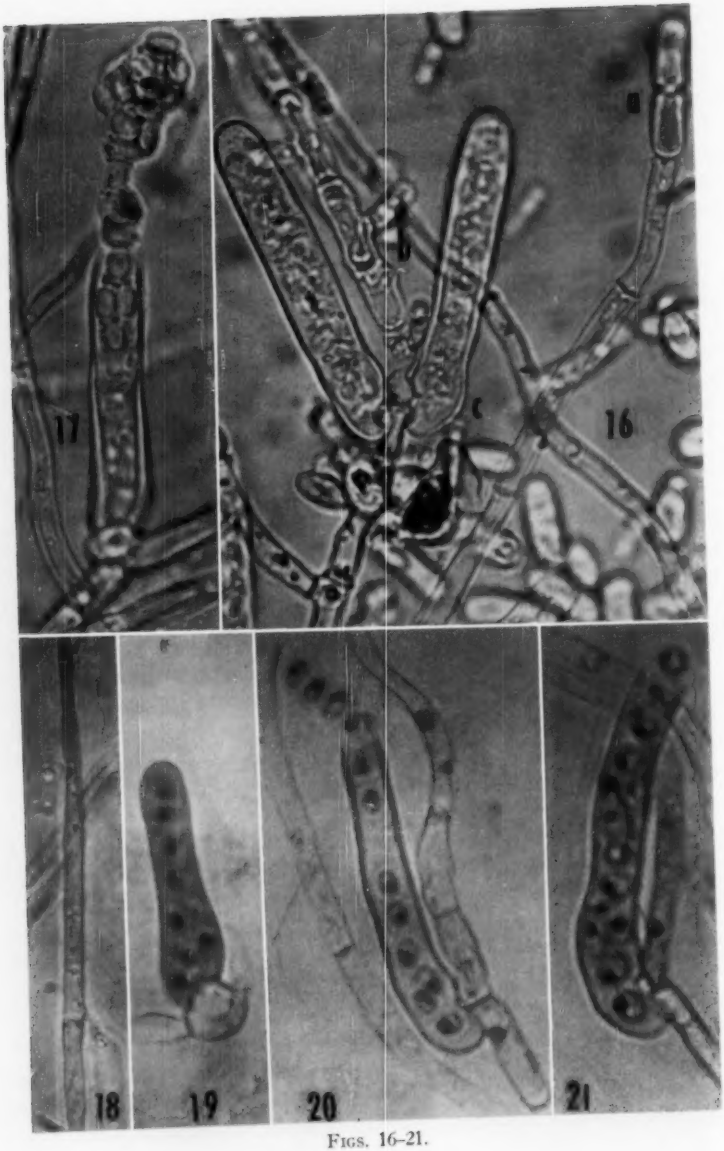
4. *Dissemination and perennation.*

a. *Ambrosia beetles and D. aggregatus.* Some of the Coleoptera have been shown to have interesting biological associations with some fungi. Several members of the family Ipsidae (Scolytidae), commonly known as "Ambrosia" beetles, feed on fungal matter, which in turn is disseminated by the beetles, as was noted by Hubbard (**1897**), Buchner (**1928, 1928b, 1953**), Leach (**1940**) and Francke-Grosmann (**1952**).

The writer has observed the association of *D. aggregatus* with *Ips pini*, an ambrosia beetle which infests the dead branches or stems of the family Pinaceae. The fragrant smell of the pure cultures and the viscid nature of the ascospores might facilitate their dispersal by *Ips pini* and other allied beetles.

The fungus has also been isolated from forest soils. Presumably the ascospores or the meristem-arthrospores are washed down by rain from the tunnels of *Ips pini*.

b. *Overwintering of the fungus.* The fungus presumably overwinters in the form of ascospores. An ascospore suspension was poured on sterilized garden soil and left out of doors. The germination of ascospores was observed periodically. The ascospores have been found to be viable for over 15 months under the temperature conditions of Ithaca, N. Y. Meristem-arthrospores under similar conditions lose viability after six months. Transfers taken from one-year-old dried agar slants stored at room temperature gave viable cultures.



FIGS. 16–21.

TAXONOMY OF THE GENUS DIPODASCUS

DIPODASCUS Lagerheim emend. Batra

Vegetative mycelium: profuse or yeast-like, septate, branched, aerial and submerged in the substratum and with cells uninucleate or multinucleate, heterotrophic with respect to biotin, or biotin and thiamin (according to Dulaney and Grutter **1950**), nitrate reduction positive or negative. *Odor:* sweet and fragrant. *Asexual reproduction:* by the production of meristem-arthrospores or yeast-like sprout cells; chlamydospores present or absent; needle-shaped synnemata present or absent. *Sexual reproduction:* homothallic; mature gametangia morphologically distinct from the mycelium or vegetative-branch initials; gametangial initials arising from contiguous cells, cut off from the gametangiogenic cells by septa to form gametangia which are conspicuous or inconspicuous, isogamous or anisogamous, uninucleate or multinucleate; ascogenous hyphae and ascocarps lacking. *Asci:* formed directly by the fusion of two gametangia, conical or cylindrical, ∞-spored, with a wall of uniform thickness throughout or slightly thickened at the apex, an apical pore smaller in diameter than the ascospores formed by the gelatinization of the wall, which sometimes becomes reflexed near the extreme tip of the empty ascus, the pore not bluing in iodine solution. *Ascospores:* unicellular, hyaline, smooth-walled, usually ellipsoid, sometimes polygonal when tightly packed in the ascus, or rarely globose and with a single eccentric nucleus, surrounded by a gelatinous sheath or capsule, and when discharged usually forming a ball or cirrus in a gelatinous substance.[12]

Habitat: saprophytic, in slime flux, soil, or associated with insects or annelids. Name: from Greek; δις = double; ποῦς = foot; ἀσχός = ascus.

A KEY TO THE SPECIES OF DIPODASCUS

A. Meristem-arthrospores present; gametangia anisogamous and conspicuous; mycelium profuse, producing needle-shaped synnemata which give a woolly and tomentose appearance to the colony; nitrate reduction absent; requiring biotin.

[12] The term used by Lagerheim (**1892**) to designate this substance was "Zwischensubstanz."

FIGS. 16–21. *D. aggregatus, photomicrographs* (× 1450). FIG. 16. a, Formation of meristem-arthrospores. b, Gametangiogenic cells. c, Two asci arising from a pair of contiguous gametangiogenic cells as a result of the fusion of the respective gametangia. FIG. 17. Ascospore discharge from a 24-spored ascus, ascospores forming a mucilaginous ball near the tip of the ascus. FIG. 18. Vegetative hypha showing reserve food particles. FIG. 19. Eight-spored ascus arising from the fusion of two very small gametangia, one not in focus. FIG. 20. Ascospore discharge from 12-spored ascus. FIG. 21. Twelve-spored ascus about to discharge ascospores with eccentric nuclei.

B. Asci cylindrical, aggregated; ascospores completely filling the asci; gametangia usually more than one per gametangiogenic cell; meristem-arthrospores, hyphal cells, and gametangia uninucleate. ..... *D. aggregatus*

BB. Asci conical, narrowly tapering towards the apex, solitary; ascospores sparsely distributed and not completely filling the asci; gametangia not more than one per gametangiogenic cell; meristem-arthrospores, gametangia, and hyphal cells multinucleate..... *D. albidus*

AA. Meristem-arthrospores absent; gametangia isogamous, inconspicuous, not more than one per gametangiogenic cell; mycelium scanty and yeast-like; tomentum absent; nitrate reduction positive; requiring biotin or both biotin and thiamin. Asci conical, narrowly tapering towards the apex, solitary; ascospores only in the upper $\frac{1}{3}$ to $\frac{1}{2}$ of the ascus, not amyloid; gametangia and mycelial cells uninucleate.. *D. uninucleatus*

DIPODASCUS ALBIDUS Lagerheim Jahrb. Wiss. Bot. **24**: 15. 1892. FIG. 1b

Morphological characters. Mycelium: profuse, gelatinous, aerial and submerged in the substratum; colony with a cottony margin and becoming gradually effuse, wet and gelatinous from the center outward, white, pale yellow according to Kobayasi (**1952**) or fawn colored with age; hyphae slender, branched, branches almost always arising near the septa; cells of the hyphae multinucleate, 25–100 × 3–6 μ. *Synnemata:* present under certain conditions. *Asexual reproduction:* by the formation of multinucleate meristem-arthrospores borne in chains; meristem-arthrospores cylindrical with truncate or rounded ends, hyaline, granular, (6–)8–30(–50) × (3–)4.5–6.5 μ. *Endospores:* formed in hyphal cells, rare, thick-walled, rectangular, pale yellow, 7–20 × 4–7 μ. *Sexual reproduction:* homothallic, by the formation and fusion of two conspicuous, anisogamous gametangia, usually arising from contiguous gametangiogenic cells or sometimes from different hyphae; gametangia solitary, multinucleate, only one produced from each gametangiogenic cell. *Asci:* rarely formed, solitary, elongate, narrowly tapering towards the apex, bifurcate at the base, with a wall of uniform thickness throughout, ∝-spored, much broader near the base, 180–250 × 15–20 μ near the base, tapering to 3–4 μ broad towards the apex. *Ascospores:* ellipsoid or subglobose, with a sticky capsule, giving a nonamyloid reaction, with a single eccentric nucleus, lying loosely in a multiseriate fashion except in the narrow neck of the ascus where they are uniseriate, (1.6–)2–3.5 × 1–2.5 μ without the capsule and (2–)2.5–5 × 2.5–3.5 μ with the capsule, discharged one by one in a gelatinous material to form a ball attached at the ascus apex.

Physiological characters. Fermentation: positive for sucrose and maltose; negative for dextrose, lactose and galactose. *Carbon assimilation:* both succinic acid and mannitol assimilated strongly. *Nitrate re-*

duction: absent. *Acid production:* none. *Growth in vitamin-free media:* none; requiring biotin.

Habitat: slime flux and exudations from the stumps of trees; *Puya* (Bromeliaceae), *Betula* (Betulaceae), *Fagus* (Fagaceae), *Cercidiphyllum japonicum* Sieb. et Zucc. (*Trochodendraceae*). Name: from Latin, *albidus* = white. Type locality: Pululahua, Pichincha, Equador.

Type specimen: Since the original material upon which Lagerheim based his species (and the genus) is probably no longer in existence, the writer designates a dried culture of Juel's isolate of *D. albidus,* CUP-A [13] 20140, as the NEOTYPE specimen.

Illustrations: [14] Lagerheim, Jahrb. Wiss. Bot. **24**: *pl. 24–26.* 1892; Ferry, Rev. Mycol. **16**: *pl. 140.* 1894; Schröter in Engler & Prantl, Pflanzenfam. I **1**(1): *fig. 129.* 1897; Juel, Flora **91**: *pl. 7, 8.* 1902; Dangeard, Le Botaniste **9**: *fig. 4.* 1904; and **10**: *pl. 1, 2.* 1907; Claussen, Botan. Zeit. **53**: *fig. 5.* 1905; Juel, Rev. Mycol. **28**: *pl. 240, fig. 1–8.* 1906; Delacroix and Maublanc, Maladies des Plantes Cultivées (Maladies Parasitaires), ed. 1, *pl. 44, fig. 7* (fide Saccardo Syll. **19**: 619. 1910, *n.v.*). 1908–09; Atkinson, Ann. Mo. Bot. Gard. **2**: *fig. 1, a–g.* 1915; Delacroix and Maublanc, Maladies des Plantes Cultivées (Maladies Parasitaires), ed. 2, *pl. 45, fig. 7, a–c.* 1916; Atkinson, College Text Book of Botany, *fig. 251.* 1908; Fitzpatrick, The Lower Fungi, *fig. 112, e.* 1930; Kobayasi, Jap. Jour. Bot. **25**: *fig. 1, 2.* 1950; Kobayasi (Kobayashi), Nat. Sci. Mus. (Tokyo) Bull. **33**: *fig. 2, 3.* 1953.

Distribution: South America (Equador, Colombia), Europe (Sweden), Asia (Japan).

Exsiccati: none.

Specimens examined: Sweden; Juel's dried cultures in CUP-A 20140, the NEOTYPE. Microscopic slides prepared by H. S. Jackson in 1905 from Juel's isolate, filed as CUP-A 20167; CUP-2092; CUP-2093. Colombia: *G. W. Martin 3855,* collected in 1935. Japan: Kobayasi, Y. Nagao Institute "N.I. 2087" (living culture). From Nippara, Tokyo. 1950; Kobayasi, Y. CUP-44732. (Material fixed in picric acid and formalin). From Nippara, Tokyo. 1950; NRRL (living culture), their number B-4352 which originally came from Japan.

Notes: All attempts to obtain asci in the cultures were unsuccessful and therefore the results reported here are partially based on preserved material. Kobayasi (**1953a**) reported that there was no fermentation of glucose, galactose, maltose, saccharose and lactose by *D. albidus.*

[13] Atkinson Herbarium, Cornell University, Dept. of Plant Pathology.

[14] No attempt has been made to list the illustrations from text-books unless reported to be original.

The writer, however, has found that it ferments saccharose and maltose, but poorly.

Lagerheim (**1892**), in his *plate 25, fig. 16,* has depicted two asci arising from a single pair of gametangiogenic cells with no mention of the condition in the text of his article. In preparations of material from Juel's isolate, and from collections of Martin, and Kobayasi, the writer has been unable to observe this condition in *D. albidus.*

DIPODASCUS UNINUCLEATUS Biggs, Mycologia **29**: 41. 1937. FIG. 1c, 3

Morphological characters. Colony: "Colony circular pulvinate, edge wavy, surface glistening, gyrose wrinkled" (Biggs **1937**). *Mycelium:* yeast-like, not profusely branched, aerial, "Pinkish Buff" to "Light Pinkish Cinnamon," hyphal cells drum-shaped, constricted near the septa, uninucleate, 10–30 × 6–12 μ. *Synnemata:* absent. *Asexual reproduction:* by yeast-like sprout cells, or by the breaking of hyphae into small units. *Sexual reproduction:* homothallic, by the fusion of two isogamous gametangia; gametangia inconspicuous, uninucleate, never more than one from each gametangiogenic cell. *Asci:* ∞-spored, solitary, narrowly tapering towards the apex, maturing from the tip downward and of very variable dimensions and wall slightly thickened at the apex, on agar 60–220 × 7–8 μ, tapering to 3–4 μ broad towards the apex, in liquid cultures reaching a length of 1 mm. *Ascospores:* ellipsoid, some spores in the asci appearing slightly allantoid, becoming globose after liberation from the asci, without a conspicuous sheath or capsule, hyaline, nonamyloid, with a single eccentric nucleus, multiseriate, 4 × 0.5–2.5 μ, sometimes budding in the ascus, gradually discharged, forming a cirrus or ball in a mucilaginous substance near the ascus apex.

Physiological characters. Fermentation: negative for dextrose, sucrose, maltose, lactose and galactose. *Carbon assimilation:* succinic acid strongly, and mannitol poorly, assimilated. *Nitrate reduction:* positive. *Acid production:* positive. *Growth in vitamin-free media:* none, requiring biotin. Dulaney & Grutter (**1950**) report a requirement also for thiamin in their subisolate of Dr. Biggs's isolate.

Habitat: saprophyte on dead pupa of *Drosophila melanogaster* Meigen. Name: from Latin, *unus* = one; *nuc-leus* (= diminitive of *nux, nucis*) = a nut, referring to the uninucleate condition of cells in the organism. Type locality: Toronto, Ontario, Canada.

Type specimen: TYPE. Slides in TRTC; isotype slides CUP-44736 and Harvard University Herbarium. Subisolates of the original cultures are in Centraal-bureau voor Schimmecultures, Baarn, Holland, and in American Type Culture Collection, Chicago, Illinois.

Illustrations: Biggs, Mycologia **29**: *figs. 1–50.* 1937.

Distribution: Canada (Toronto), U. S. A. (Berkeley, California).

Exsiccati: none.

Specimens examined: isotype slides; subisolates of type culture; and NRRL y-2181.

Notes: Except for terminal asci, most of the observations made by Biggs (**1937**) were confirmed. In addition, it was observed that the organism produces yeast-like sprout cells which serve as asexual reproductive bodies. Occasionally such sprout cells may conjugate, with or without the formation of characteristic beak-like gametangia, and give rise to few-spored asci. Measurements of ascospores reported by the writer are larger than those given by Biggs (**1937**).

Some very long asci observed in liquid cultures were branched and both the trunk as well as the branches of the ascus contained ascospores. Such asci were formed as a result of the fusion of two gametangia.

Ascospores in Dr. H. J. Phaff's isolate (NRRL y-2181) are slightly larger and appear to be more curved (more or less allantoid) than those of other isolates. This isolate differs biochemically from other isolates of *D. uninucleatus* in being "incapable of assimilating the fructose-containing carbohydrates sucrose, raffinose and inulin" (personal communication from Dr. L. J. Wickerham of USDA). This isolate can utilize D-arabinose and rhamnose but other isolates do not.

The yeast-like habit of the colony, asexual reproduction by sprout cells and their occasional conjugation, the nutritional requirements, and the parthenogenetic asci reported by Biggs suggest that the organism may be phylogenetically related to some members of the Saccharomycetaceae.

DIPODASCUS AGGREGATUS Francke-Grosmann, Meddel. Stat. Skogsforskninginst. **41**(6): 30. 1952, emend. Batra FIGS. 1a, 2, 4–21

= *Dipodascus albidus* forma *minor* Korf, Sydowia, Beih. **1**: 286. 1957

Morphological characters. Mycelium: profuse, slimy to gelatinous, aerial and submerged in the substratum or agar medium, white to "Pinkish Vinaceous," in age ranging from white to "Light Purplish Vinaceous"; hyphae branched, branches arising adjacent to the septa in a sympodial fashion; cells of the hyphae uninucleate, (17–)20–100 (–120) × (2.5–)4.4–5(–8.5) μ. *Synnemata:* present under certain conditions. *Asexual reproduction:* by the formation of uninucleate meristem-arthrospores borne in chains, hyaline, rectangular, with truncate ends, (3.5–)4.5–13(–15) × 1.7–2.5 μ. *Endospores:* absent. *Sexual reproduction:* homothallic, by the fusion of two conspicuous, anisogamous

gametangia, usually arising from contiguous gametangiogenic cells, gametangia uninucleate throughout and with rare exception arising in clusters of 2–5 from each gametangiogenic cell. *Asci:* aggregated in clusters, cylindrical, with a rounded apex and bifurcate base, ascus wall of uniform thickness throughout, not staining with iodine solution, pore apical, smaller than the width of the ascospores, (8–)24–32(–64) spored, 47–130 × 6–10.5 μ. *Ascospores:* ellipsoid to subglobose, becoming polygonal when tightly packed in an ascus, surrounded by a conspicuous gelatinous capsule, nonamyloid, with a single eccentric nucleus, partially or wholly uniseriate or biseriate in the ascus, (2.5–)4.2–5.5 × (2.5–)3–3.5 μ without the capsule, 5–9 × 5–6.5 μ with the capsule, discharged one by one to form a ball attached to the tip of the ascus for a considerable period of time.

Physiological characters. Fermentation: positive for dextrose, slow; negative for sucrose, maltose, lactose, and galactose. *Carbon assimilation:* succinic acid strongly, mannitol poor. *Nitrate reduction:* absent. *Acid production:* none. *Growth in vitamin-free media:* absent, requiring biotin.

Habitat: pupal galleries of *Ips acuminatus* Gyll. in *Pinus sylvestris* L.; frass of *Ips pini* Say in *Pinus resinosa* Ait. and in *Picea engelmanni* Parry, reported from the roots of *Pinus resinosa* by Korf (**1957**). Type locality: Hamra, Dalarna, Sweden.

Type specimen: Since Dr. Francke-Grosmann did not preserve a type specimen (Francke-Grosmann, personal communication) the writer hereby designates a dried culture of her isolate, CUP 44778, as the LECTOTYPE specimen.

Illustrations: Meddl. Stat. Skogsf. Inst. **41**(6): *fig. 8, 17.*

Distribution: Europe (Northern and Central Sweden. June to August). North America (New York, Wyoming. June to August).

Exsiccati: none.

Specimens examined: Sweden: LECTOTYPE from the pupal galleries of *Ips acuminatus.* U. S. A.: New York: Horseheads, CUP 43905, the TYPE specimen of *D. albidus* forma *minor,* and CUP 44260. Wyoming: Medicine Bow Mountains, NRRL y-2425.

Notes: Unlike *D. albidus,* as described by Lagerheim (**1892**), *D. aggregatus* does not form asci in advance of meristem-arthrospores. The writer could not find reniform ascospores as described by Francke-Grosmann (**1952**) for *D. aggregatus* and he believes that her microscopic preparations were contaminated with foreign spores, as the photomicrograph of an ascus by the same writer shows ellipsoid spores clearly.

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LITERATURE CITED

Alexopoulos, C. J. 1952. Introductory mycology. i–xi. 1–482. New York. John Wiley & Sons.

Bessey, E. A. 1935. A text book of mycology. i–xv. 1–495. Philadelphia. Blakiston.

——. 1950. Morphology and taxonomy of fungi. i–ix. 1–791. Philadelphia and Toronto. Blakiston.

Biggs, Rosemary. 1937. *Dipodascus uninucleatus.* Mycologia **29**: 34–44.

Brefeld, O. 1891. Die Hemiasci und die Ascomyceten. Untersuchungen aus dem Gesammtgebiete der Mykologie **9**: 94–108.

Buchner, Paul. 1928a. Ergebnisse der Symbiose Forschung. 1–129. *In* Ergebnisse der Biologie. 1–717. 293 figs. Berlin. J. Springer.

——. 1928b. Holznabrung und Symbiose (Wood nutrition and symbiosis). 1–64. 22 figs. Berlin. J. Springer.

——. 1953. Endosymbiose der Tiere mit Pflanzlichen Mikroorganismen. (Lehrbücher und Monographien aus dem Gebiete der exakten Wissenschaften.) Reihe der Experimentellen Biologie Bd. **12**: 1–771. Basel. Birkhäuser.

Carmichael, J. W. 1956. The cellophane technique for studying morphology and fusions in fungi. Mycologia **48**: 450–452.

Dangeard, P. A. 1904. Recherches sur le développement du périthèce chez les ascomycètes. Botaniste **9**: 59–158.

——. 1907. Recherches sur le développement du périthèce chez les ascomycètes. Botaniste **10**: 1–385.

Delacroix, G., and A. Maublanc. 1916. Maladies des plantes cultivées (Maladies parasitaires). 1–447. ed. 2. Paris. Baillière et Fils.

Dulaney, E. L., and F. G. Grutter. 1950. A note on the culture of *Dipodascus uninucleatus* in defined media. Mycologia **42**: 654–657.

Francke-Grosmann, Helene. 1952. Ueber die Ambrosiazucht der beiden Kiefernborkenkäfer *Myclophilus minor* Htg. und *Ips acuminatus* Gyll. Meddel. Statens Skogsforskningsinst. Stockh. **41**(6): 1–52.

Gäumann, E. 1926. Vergleichende Morphologie der Pilze. i–x. 1–626. Jena. G. Fisher.

——. 1928. Comparative morphology of fungi. Translated and revised by C. W. Dodge. i–xiv. 1–701. New York. McGraw-Hill.

——. 1952. The fungi. Translated by F. L. Wynd. 1–420. New York. Hafner Publishing Co.

Gwynne-Vaughn, H. C. I., and B. Barnes. 1927. The structure and development of the fungi. i–xvi. 1–384. Cambridge. The University Press.

Hoffmeister, E. R. 1953. Trypan blue as a nuclear stain for plant material. Stain Techn. **28**: 309, 310.

Holtermann, C. 1898. Mycologische Untersuchungen aus den Tropen. i–viii. 1–122. *12 pl.* Berlin.

Hubbard, H. G. 1897. The ambrosia beetles of the United States. U. S. Dept. Agr. Div. Ent. Bull. **7**: 9–30.

Hughes, S. J. 1953. Conidiophores, conidia, and classification. Canad. Jour. Bot. **31**: 577–659.

Johansen, D. A. 1940. Plant microtechnique. i–xi. 1–523. New York. McGraw-Hill.

Juel, H. O. 1902. Ueber Zellinhalt, Befruchtung, und Sporenbildung bei *Dipodascus*. Flora **91**: 47–55.

——. 1921. Cytologische Pilzstudien II. Zur Kenntnis einiger Hemiasceen. Nova Acta Reg. Soc. Sci. Upsal. IV. **5**(5): 1–43.

Keitt, G. W. 1915. Simple technique for isolating single spore strains of certain types of fungi. Phytopathology **5**: 266–269.

Kobayasi, Y. 1950. On the Dipodascaceae. Jap. Jour. Bot. **25**: 161–164.

Kobayasi (Kobayashi), Y. 1953a. Yeasts and molds in the trunk exudations. National Science Museum (Tokyo) Bull. **33**: 31–46.

Koch, R. 1883. Ueber die neuen Untersuchungsmethoden zum Nachweis der Mikrokosmen in Boden, Luft und Wasser. *Reprinted in* Ges. Werke von R. Koch **1**: 274–284. Berlin, 1912. (Cited from McNew, G. L., in Phytopathology **28**: 387–401.)

Korf, R. P. 1957. *Dipodascus albidus* forma *minor*. Sydowia Beih. **1**: 285–288.

Lagerheim, G. 1892. Eine neue geschlectliche Hemiascae, *Dipodascus albidus*. Jahrb. Wiss. Bot. **24**: 549–565.

——. 1894. Le *Dipodascus albidus*, nouvel Hemiascus à reproduction sexuelle (Jahrb. Wiss. Bot. **24**: 549–565). Traduct. du Dr. R. Ferry. Rev. Mycol. **16**: 45–52. *pl. 140.*

Lanjouw, J., and F. A. Stafleu. 1956. Index Herbariorum. I. The Herbaria of the world. ed. 3. Regnum Vegetabile **6**: 1–224.

Leach, J. G. 1940. Insect transmission of plant diseases. i–xi. 1–615. New York. McGraw-Hill.

Lindau, G. 1900. Hemiascineae. *In* Engler, A., und K. Prantl. Die natürlichen Pflanzenfamilien I **1**(*2*): 531.

——. 1905. Hemiasci. *In* Kryptogamenflora der Mark Brandenburg **7**(1): 1. Leipzig. Gebrüder Borntraeger.

Lodder, J., and N. J. W. Kreger-van Rij. 1952. The Yeasts, a taxonomic study. i–xi. 1–713. New York. North Holland Publishing Co.

Martin, G. W. 1937. New or noteworthy fungi from Panama and Colombia. I. Mycologia **29**: 618–625.

——. 1954. Key to the families of fungi. *In* Ainsworth, G. C., and G. R. Bisby. A dictionary of the fungi. ed. 4. i–vii. 1–475. Kew. Commonwealth Mycological Institute.

Martin, W. J. 1943. A simple technique for isolating spores of various fungi from exposed slides in aerobiological work. Phytopathology **33**: 75–76.

McNew, G. L. 1938. Dispersion and growth of bacterial cells suspended in agar. Phytopathology **28**: 387–401.

Moreau, Fernand. 1954. Les Champignons; Physiologie, Morphologie, Développement et Systématique. II. Systématique. Encyclopédie Mycologique **23**: 1259–1278.

Nannfeldt, J. A. 1932. Studien über die Morphologie und Systematik der nichtlichenisierten inoperculaten Discomyceten. Nova Acta Reg. Soc. Sci. Upsal. IV **8**(*2*): 1–368.

Pfeffer, W. 1899. The physiology of plants. Translated and edited by A. J. Ewart. ed. 2. **1**: i–xii. 1–632. Oxford. Clarendon Press.

Ridgway, R. 1912. Color standards and color nomenclature. 1–43, *illus.* Washington, D. C.

Schröter, J. 1897. Hemiascineae. *In* Engler, A., und K. Prantl. Die natürlichen Pflanzenfamilien I **1**(1): 143–149. Leipzig. W. Engelmann.

Stelling-Dekker, N. M. 1931. Die sporogenen Hefen. Verhandel. Koninkl. Akad. Wetenschap. Afd. Natuurk. II. **28**: i–vii. 1–547.

Varitchak, Bogdan. 1928. L'évolution nucleaire chez *Ascoidea rubescens* Brefeld. Compt. Rend. Acad. Sci. Paris **186**: 96–98.

——. 1931. Contribution à l'étude du développement des Ascomycètes. Botaniste **23**: 1–182.

Wickerham, L. J. 1951. Taxonomy of yeasts. U.S.D.A. Tech. Bull. **1029**: 1–56.

——, **and K. A. Burton.** 1948. Carbon assimilation tests for the classification of yeasts. Jour. Bact. **56**: 363–371.

White, W. L. 1942. A new hemiascomycete. Can. Jour. Res. C **20**: 389–395.

Wolf, F. A., and F. T. Wolf. 1947. The fungi. Vol. I. i–x. 1–438. New York. John Wiley & Sons.

WOOD EXTRACT AND GROWTH OF MORCHELLA

WILLIAM J. ROBBINS AND ANNETTE HERVEY

(WITH 2 FIGURES)

Brock has reviewed the literature on the nutrition of *Morchella esculenta* Fr. and reported his own findings (**2**). The minimum nutritional requirements of this fungus are relatively simple. It grows quite well at neutral reaction in a mineral-salt-glucose medium with nitrate as the sole source of nitrogen. Brock found starch, maltose, fructose, turanose and sucrose, as well as dextrose, to be good sources of carbon, and cysteine-HCl, aspartic acid, asparagine, urea, nitrite, ammonium salts and glutamic acid, in addition to nitrate, good sources of nitrogen. A neutral or slightly alkaline reaction was found by Fron (**6**) and Brock (**2**) to be more favorable for growth than more acid or more alkaline reactions. Although the nutritional requirements for the growth of *M. esculenta* are simple, the conditions for the formation of sporophores are not defined and their production in culture has been only rarely reported.

The favorable action of wood, tomato or malt extract on the growth of some of the higher fungi has been reported from this laboratory previously (**9, 10, 11, 12**). The present paper concerns the effect of these natural substances, especially a water extract of dry beech wood, on the growth of *Morchella esculenta* Fr. and *Morchella crassipes* Fr.

MATERIALS AND METHODS—The culture of *M. crassipes* was kindly furnished by Dr. W. D. Gray; that of *M. esculenta* was a tissue culture prepared from a sporophore in our laboratories. The basal media included medium (A), Brock's medium, which contains mineral salts and dextrose with nitrogen furnished as $NaNO_3$, and medium (B), a complex medium of mineral salts, dextrose, vitamins of the B-complex, purine and pyrimidine bases, and "vitamin-free" casein hydrolysate which had been neutralized with $CaCO_3$ (see reference **11** for composition).

The fungi were grown as still cultures in 25 or 30 ml of liquid medium in 125 ml Erlenmeyer flasks or in 10 ml of medium in test tubes. Cultures were inoculated from colonies grown on 2 per cent malt-extract

TABLE 1

DRY WEIGHT PER CULTURE OF MORCHELLA ESCULENTA GROWN AT 25° IN 25 ML OF A MINERAL-SALT-DEXTROSE MEDIUM WITH $NaNO_3$ AS NITROGEN SOURCE AND IN SAME MEDIUM SUPPLEMENTED WITH MINOR MINERAL ELEMENTS, B VITAMINS, PURINE AND PYRIMIDINE BASES AND WOOD EXTRACT

Additions to Medium (A) per flask	MEDIUM (A) ACID					MEDIUM (B) NEUTRAL				
	Initial pH	After 1 week		After 2 weeks		Initial pH	After 1 week		After 2 weeks	
		Final pH	Dry wt. mg	Final pH	Dry wt. mg		Final pH	Dry wt. mg	Final pH	Dry wt. mg
None	5.0	5.7	3	6.45	17	7.1	7.2	7	7.6	24
Supplements of Medium (B)	4.6	5.2	5	6.75	33		7.15	9	7.6	32
Supplements & 1 mg wood ext.	4.5	5.7	8	6.8	36	7.1	7.15	15	7.7	41
Supplements & 5 mg wood ext.		6.2	14	7.15	52		7.1	14	7.7	51
Supplements & 25 mg wood ext.	4.3	6.3	16	7.7	122	6.8	7.0	16	7.9	129

agar by the method of Yusef (**16**) and were incubated at 25° C. The inoculum consisted of a piece of mycelium about 1 mm in diameter. Although cultures were inoculated as described, more rapid growth could be obtained if bits of mycelium from liquid cultures of (B) medium were used as inoculum. Growth was more rapid at 25° than at 20°. Dry weights were determined by treating the mycelium with ethanol, filtering into Gooch crucibles, washing with 70 per cent ethanol and drying at 100°. The wood and tomato extracts were prepared as described previously (**11**).

PH AND MORCHELLA—Fron and Brock found a neutral or slightly alkaline reaction more favorable for the growth of *M. esculenta* than acid

TABLE 2

DRY WEIGHT PER CULTURE OF M. ESCULENTA GROWN AT 25° IN 25 ML OF A MINERAL-SALT-DEXTROSE MEDIUM SUPPLEMENTED WITH MINOR MINERALS, B VITAMINS, PURINE AND PYRIMIDINE BASES. SOURCE OF NITROGEN WAS CASEIN HYDROLYSATE

Additions to Medium (B) per flask	MEDIUM (B) ACID					MEDIUM (B) NEUTRAL				
	Initial pH	After 1 week		After 2 weeks		Initial pH	After 1 week		After 2 weeks	
		Final pH	Dry wt. mg	Final pH	Dry wt. mg		Final pH	Dry wt. mg	Final pH	Dry wt. mg
None	4.3	4.35	0.2	4.35	0.5	6.5	6.2	11	5.9	89
1 mg wood extract	4.2	4.1	2	3.5	40	6.5	6.0	20	6.0	196
5 mg wood extract	—	3.9	9	3.4	107	—	6.05	16	6.1	225
25 mg wood extract	—	5.1	7	3.6	147	6.4	6.0	20	6.2	242

reactions. Our results support their conclusions. For example, we obtained 3 mg of mycelium per culture in one week and 17 mg in two weeks in medium (A) which had been acidified with HCl to initial pH 5.0. But at initial pH 7.1 the dry weight in one week was 7 mg and in two weeks 24 mg (TABLE I). In our medium (B) at initial pH 4.3 almost no growth occurred, but when medium (B) was adjusted to initial pH 6.5 with NaOH, we obtained 11 mg in one week and 89 mg in two weeks (TABLE II).

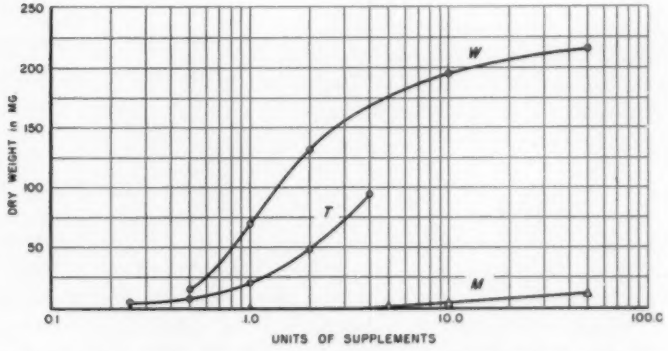

FIG. 1. Dry weight per culture of *Morchella esculenta* plotted against additions of wood (W), tomato (T) or malt extract (M) to a mineral-salt-dextrose medium at pH 4.5 supplemented with B vitamins, purine and pyrimidine bases and containing casein hydrolysate. 1 unit wood extract = 0.5 mg per flask (25 ml); 1 unit tomato extract = 45.1 mg per flask; 1 unit malt extract = 1 mg per flask.

The fungus grew better in medium (A) at an initial acid reaction (pH 4.5–5.0) than in acid medium (B). This is probably because as growth occurred in medium (A), which contains $NaNO_3$, the reaction became less acid; this did not occur in medium (B), where nitrogen was furnished as casein hydrolysate.

EXTRACTS AND MEDIUM (B)—Growth of *M. esculenta* in medium (B) at pH 4.5 was slight, amounting in 14 days to a few mg at most. The addition of wood extract, tomato juice or malt extract to this medium had little effect on the hydrion concentration, but improved growth. Wood extract was especially effective; malt extract, least effective.

For example, growth in two weeks at 25° in acid medium (B) amounted to 2 mg per culture. The addition of 0.25 mg (10 μg per ml of medium) of wood extract per flask increased it to 15 mg and with

25 mg of extract 217 mg of mycelium were obtained. The pH in the (B) medium at the end of 14 days growth was 4.3 and with 25 mg of wood extract it was pH 4.4. With the largest amount of tomato extract, 4.0 units per flask, 94 mg dry weight of mycelium were obtained and the final pH was 4.3. With 50 mg of malt extract per flask, 13 mg of mycelium developed and the final pH was 4.0 (FIG. 1).

The response of *M. crassipes* to the extracts was much as described for *M. esculenta*. We observed, however, that *M. crassipes* formed sclerotia on 2 per cent malt agar; *M. esculenta* did not. Wood extract not only affected growth favorably in medium (B) at initial pH 4.3 to 4.5, but also in the same medium of initial pH 6.4 to 6.5 (TABLE II).

WOOD EXTRACT AND MEDIUM (A)—Wood extract was beneficial when added in amounts ranging from 0.25 mg to 2.5 mg per flask to medium (A) of initial pH 4.8 or 6.8. However, the addition to medium (A) at these initial reactions of the mineral supplements, B vitamins and the purine and pyrimidine bases of medium (B) increased growth to the same order of magnitude as the wood extract; thiamine used alone as a supplement to medium (A) was ineffective (compare Hurni). We therefore examined the effect of wood extract in medium (A) to which the supplements of medium (B) were added. Under these conditions wood extract was beneficial at acid and neutral reactions. There was little difference in the effect of the extract in the acid or neutral medium (TABLE I). Growth in the less acid medium (B) supplemented with wood extract was superior to that obtained in the supplemented medium (A) containing wood extract.

DILUTION CULTURES—The favorable effect of the addition of wood extract to acid medium (B) was confirmed for both *M. esculenta* and *M. crassipes* by dilution cultures. Test tubes 20 × 150 mm and 25 × 150 mm each containing 10 ml of medium were used with similar results. The first tube of each series contained 5 mg of wood extract and dilution by a factor of 2 was employed. When the growth in the tubes was examined with a 10 × hand lens, the effect of 0.312 mg of wood extract per tube was evidenced by new growth from the inoculum within 24 hours after inoculation. After seven days growth was greatest in the tube containing 5 mg of wood extract and decreased to tube 5 which contained 0.312 mg. A sharp difference between the growth in tube 5 and tube 6 was evident (FIG. 2). Wood extract increased the growth of *M. esculenta* in acid and neutral medium (A) when tested in dilution tubes. The initial reactions were pH 4.3 for the acid series and pH 6.8 for the neutral series. No improvement was noted from the addition of $NaNO_3$ or $(NH_4)_2SO_4$ to the first tube of a dilution series with

medium (B). This indicates that the effect of wood extract is not due to its nitrogen content as such.

NATURE OF BENEFICIAL MATERIAL—The material present in wood and tomato extract which improved the growth of *M. esculenta* and *M. crassipes* was water soluble, thermostable and non-volatile. It was

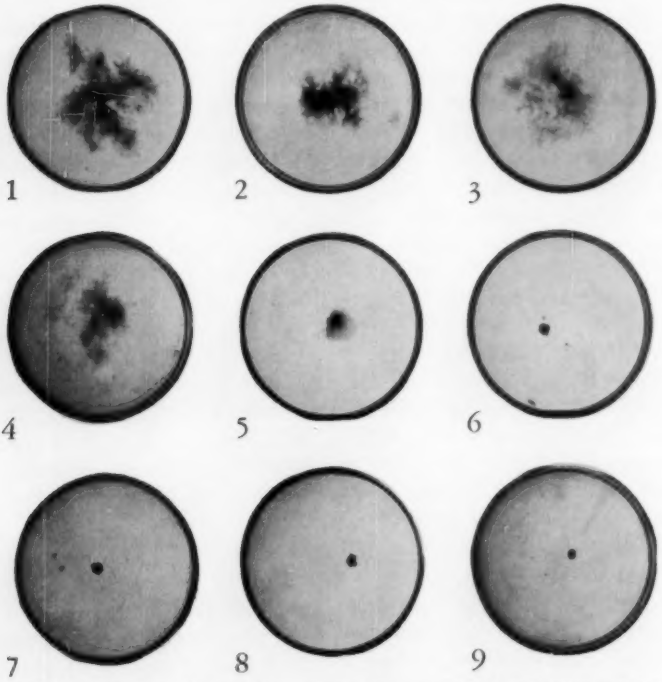

FIG. 2. Growth of *Morchella crassipes* after one week in test tubes containing basal medium of mineral salts, dextrose, B vitamins, purine and pyrimidine bases and casein hydrolysate at pH 4.5 to which various amounts of wood extract were added as follows: 1) 5.0 mg wood extract; 2) 2.5 mg; 3) 1.25 mg; 4) 0.625; 5) 0.312; 6) 0.156; 7) 0.078; 8) 0.039; 9) 0.019. The contents of the test tubes were poured into petri dishes for photographing.

not soluble in chloroform or ethyl acetate. When wood or tomato extract was shaken with norit A most of the activity was found in the filtrate and little was present in the eluate prepared by treating the charcoal with ammoniacal acetone. However, the combination of eluate and filtrate was more effective than either alone, which suggests the possi-

bility of multiple factors. It is not identical with the B-vitamins nor the purine and pyrimidine bases of our (B) medium. Its activity is not due to its effect on the reaction of the medium. We have not excluded the possibility that amino acids in wood extract account for its beneficial effect on the growth of *Morchella*.

Robbins and Ma (**12**) found a strain of *Trichophyton mentagrophytes* unable to use ammonia and to grow more rapidly on a mixture of amino acids than on asparagine. According to Fries (**5**), the growth of *Lentinus onphalodes* and several other Basidiomycetes is improved by tryptophane. Methionine is required by some aquatic phycomycetes (**1, 8**). Fraser and Fujikawa (**3**) report that phenylalanine, proline and methionine improved the growth of *Agaricus bisporus*.

That known amino acids account for our results seems unlikely, however, because our medium (B) contains casein hydrolysate and a positive effect was observed on the addition of 0.25 mg of wood extract to 25 ml of medium. The quantity of any amino acid in this amount of extract must be but a small fraction of the total. Further work is needed to identify the factor(s) concerned.

DISCUSSION—Our investigation raises several questions. Why, for example, does *Morchella* grow so well at neutral reaction? Many organisms suffer from an unavailability of heavy metals as the reaction of the medium approaches neutrality. Does *Morchella* have a lower requirement for Fe, Mn and other minor elements than organisms which experience difficulty in obtaining adequate amounts of one or more of these elements from media of neutral reaction? Does *Morchella* have a more effective mechanism for absorbing the mineral elements from a neutral medium than many other organisms have?

Whatever the answer to these questions may be, it is unlikely that it will explain also why the fungus grows so poorly in acid medium, either a simple mineral-salt-dextrose medium with $NaNO_3$ as the nitrogen source, or in a mineral-salt-dextrose medium supplemented with a variety of growth substances and with casein hydrolysate as a source of nitrogen.

The poor growth in acid medium would not seem to be due to the toxicity of the H ion per se or to that of some other constituent of the medium. This follows because the marked beneficial action of wood extract, for example, was evidenced in solutions which were initially acid and remained so throughout the growth period. The favorable action of small amounts of wood extract suggests also that it is not active by neutralizing or antagonizing some toxic material in the acid medium.

We believe that wood extract and other natural substances supply *Morchella* with an unidentified growth substance (or more than one) for which the organism is partially heterotrophic and that this substance is not included in the various supplements present in our (B) medium. If this hypothesis is correct, then we might assume further that the unidentified material is synthesized to some extent by the fungus at neutral reaction, but is not synthesized when the medium is acid. We failed, however, to demonstrate the presence in the mycelium of *Morchella* of substances favorable to its own growth. The addition to our medium (B) of water extracts of *M. esculenta* grown in medium (A) had no effect on its growth. Perhaps the hypothetical growth substance(s) may be used as rapidly as synthesized.

Observations have been reported by others which suggest that the reaction of the medium influences the synthesis of an essential metabolite. Stokes, Foster and Woodward (**14**) and Strauss (**15**) found that a pyridoxine-less strain of *Neurospora* synthesized the vitamin at pH 5.8 and above in the presence of ammonia. Srb and Horowitz (**13**) report that an arginine-less *Neurospora* grew at pH 5.5 when supplied with citrulline or arginine but did not grow in the presence of ornithine. At pH 6.7 or 8.0 it utilized ornithine. It might be suggested that for this organism the enzyme systems concerned in the transformation of ornithine to citrulline were inactive when the medium was acid but became active at neutrality. Fries (**4**) observed that *Mitrula paludosa* did not grow at pH 3.6 or 5.8 unless supplied with biotin when the nitrogen source was NH_4Cl. However, with KNO_3 as a source of nitrogen the fungus grew without a supply of biotin at pH 3.6, but not at pH 5.8.

We have previously discussed the probability that unidentified growth substances account for the favorable effect of natural preparations on the growth of various of the higher fungi. Our experiments suggest that *Morchella* also suffers from a partial deficiency for unidentified growth substances. The identity of the factors concerned in the growth of *Morchella* might be determined with greater facility than with some of the slower-growing fungi previously discussed (**11**) since a 24- or 48-hour assay could probably be developed for *Morchella*.

SUMMARY

Growth of *Morchella esculenta* and *M. crassipes* in a medium of mineral salts, dextrose, casein hydrolysate, B vitamins and purine and pyrimidine bases at pH 4.5 is slight. It is markedly increased by the addition of natural preparations, especially wood extract. Wood extract is effective also at neutral reactions. The presence in natural material

of an unidentified growth substance (or substances) for which *Morchella* is partially deficient is suggested, and some characterization of the substance(s) is given.

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LITERATURE CITED

1. **Barner, H. D., and E. C. Cantino.** 1952. Nutritional relationships in a new species of *Blastocladiella*. Am. Jour. Bot. **39**: 746–751.
2. **Brock, Thomas D.** 1951. Studies on the nutrition of *Morchella esculenta* Fries. Mycologia **43**: 402–422.
3. **Fraser, Ian M., and Byron S. Fujikawa.** 1958. The growth-promoting effect of several amino acids on the common cultivated mushroom, *Agaricus bisporus*. Mycologia **50**: 538–549.
4. **Fries, Nils.** 1943. Die Einwirkung von Adermin, Aneurin, und Biotin auf das Wachstum einiger Ascomyceten. Symb. Bot. Upsal. **7** (2): 1–73.
5. ——. 1950. Precursors of tryptophan in the nutrition of *Lentinus omphalodes* Fr. and some other Hymenomycetes. Physiol. Plant. **3**: 185–196.
6. **Fron, G.** 1905. Sur les conditions de développement du mycélium de la morelle. Compt. Rend. Acad. Sci. Paris **140**: 1187–1189.
7. **Hurni, Hans.** 1946. Zur Physiologie von *Morchella esculenta*. Ihre Aneurinsynthese unter verschieden Bedingungen. Mitteil. Naturforsch. Gesell. Berne II **4**: 11–18.
8. **Machlis, L.** 1953. Growth and nutrition of water molds in the subgenus *Euallomyces*. I. Growth factor requirements. Am. Jour. Bot. **40**: 189–195.
9. **Robbins, William J.** 1950. A survey of the growth requirements of some Basidiomycetes. Mycologia **42**: 470–476.
10. ——, **and Annette Hervey.** 1955. Growth substance requirements of *Stereum murraii*. Mycologia **47**: 155–162.
11. —— **and** ——. 1959. Wood, tomato and malt extracts and growth of some Basidiomycetes. Mycologia **50**: 745–752.
12. ——, **and Roberta Ma.** 1945. Growth factors for *Trichophyton mentagrophytes*. Am. Jour. Bot. **32**: 509–523.
13. **Srb, Adrian M., and N. H. Horowitz.** 1944. The ornithine cycle in *Neurospora* and its genetic control. Jour. Biol. Chem. **154**: 129–139.
14. **Stokes, J. L., J. W. Foster, and C. R. Woodward.** 1943. Synthesis of pyridoxin by a pyridoxin-less X-ray mutant of *Neurospora sitophila*. Arch. Biochem. **2**: 235–245.
15. **Strauss, Bernard.** 1951. Studies on the vitamin B_6-requiring, pH sensitive mutants of *Neurospora crassa*. Arch. Biochem. **30**: 292–305.
16. **Yusef, Hasan M.** 1953. The requirements of some Hymenomycetes for essential metabolites. Bull. Torrey Bot. Club **80**: 43–64.

SUBTERRANEAN ELAPHOMYCES AND RHIZOPOGON IN THE MICHIGAN JACK-PINE REGION [1]

WILLIAM T. GILLIS

(WITH 3 FIGURES)

In the autumn of 1955, two deer hunters brought specimens of subterranean fungi to the Department of Botany and Plant Pathology at Michigan State University, reporting that deer were seen digging them from "bottom lands" in the northern part of the Lower Peninsula of Michigan. Upon subsequent investigations, I found that the fungi (*Elaphomyces* and *Rhizopogon*) could be located readily at places where deer hoof marks gave evidence of shallow digging (FIG. 1). Most collections of *Elaphomyces* have been made when accompanying parasites, *Cordyceps* spp., protrude above the ground (Mains, **1957**). However, none of the specimens seen in the field were thus parasitized, the deer diggings alone indicating their presence. It is interesting to note here that the common name for the Elaphomycetaceae is "deer fungi." Three species were identified by Dr. Carroll W. Dodge, to whom I owe sincere appreciation: *Elaphomyces granulatus* Fries and *E. anthracinus* Vitt. (Plectascales), and *Rhizopogon roseolus* (Corda) Hollós (Hymenogastrales).

Specimens were collected in the imperfectly drained Au Gres and moderatedly well drained Croswell sands, and were invariably at the interface between the organic layer and the mineral horizon, which, in these podzols, is quite distinct. The vegetation is largely blueberry, sweet fern, and mixed grasses with an overstory of jack pine and black oak (FIG. 2). Sections were made through the fruiting bodies and were preserved in lactophenol. Specimens and slides are deposited in the herbarium of Michigan State University.

The species collected in the greatest abundance was *Elaphomyces granulatus*. All specimens had a cinnamon, verrucose peridium. The asci had evanesced and the black spores were between 24.6 and 32.6 μ in diameter. Previous collections of this species in Michigan have been

[1] Publication No. 58-18, Department of Botany and Plant Pathology, Michigan State University.

made only in deciduous forests. The locality data for the specimens are: *Gillis 56-6a,* Oscoda Co., Mich., T. 25N, R. 1E, Sect. 20, 24 July 1956; *Gillis 56-7,* Roscommon Co., Mich., T. 24N, R. 2W, Sect. 9, 31 July 1956; *Cantlon & Byer 58-001,* Roscommon Co., Mich., T. 25N, R. 1W, Sect. 9; *Cantlon & Byer 58-002,* Crawford Co., Mich., T. 25N, R. 3W, Sect. 29; *Cantlon & Byer 58-003,* Crawford Co., Mich., T. 25N, R. 1W, Sect. 9, all 17 May 1958. Some trees in the Oscoda County collection site have been cut since the 1956 collections, but specimens of the fungus were still found easily in November 1957.

Fries recognized the name *Elaphomyces granulatus* for this species in Vol. III of Systema Mycologicum (**1829**). Dodge (**1929**) pointed out that the epithet *cervinus* has been in use since the sixteenth century. However, the Linnaean species *Lycoperdon cervinus* was a mixture of *Scleroderma* and *Elaphomyces.* Fries (**1829,** p. 59) provided the name *E. granulatus* because *E. cervinus* was a "nomen dubium," being applied to two different organisms. Dodge (**1929**) recognized the name *E. cervinus* given by Schlectendahl, as derived from the name of Persoon (**1801**), on the basis of priority. However, even though this fungus was originally thought to be a basidiomycete, with its nomenclature stemming from Persoon, the work of Fries is indeed the proper authority under Art. 13 and par. f of the International Code of Botanical Nomenclature (Lanjouw et al., **1956**). *Elaphomyces granulatus* Fries is thus the correct name. According to Dodge (**1929** and recent personal communication), Fries changed the name because of the obscene folklore associated with the species: that sperm from deer masturbations gave rise to the fruiting bodies, and that it could be used as an aphrodisiac. Fries was aware of this fable, but in justifying his use of *E. granulatus,* he mentioned only the confusion associated with the Linnaean species.

One specimen of *Elaphomyces anthracinus* was collected, but was damaged in the process. This material has been preserved therefore only in the form of slide mounts. The asci and ascospores in this mount are in various stages of development. The final spore size is 24–25 μ, slightly larger than reported in Dodge's monograph (**1929**) (FIG. 3). The fructification is not quite mature, and the characteristic dark zone between the cortex and the gleba is not well defined; the peridium is black. This fungus previously has been reported from Central Europe and in the Western Hemisphere only from the mountains of North Carolina and Tennessee (Dodge, **1929** and recent personal communication). Locality data for this specimen are: *Gillis 56-6c,* Oscoda Co., Mich., T. 25N, R. 1E, Sect. 20, 24 July 1956. Labels on specimens

FIGS. 1–3.

examined from the Farlow Herbarium indicate that this species is host for *Cordyceps capitata* in Tennessee.

Rhizopogon roseolus was colored like *Elaphomyces granulatus*, but it also was damaged in collection, inasmuch as the two damaged specimens were growing together. The extensive rhizomorphs commonly associated with *Rhizopogon* (Bessey, **1950**) were not seen. Locality data for this specimen are: *Gillis 56-6b*, Oscoda Co., Mich., T. 25N, R. 1E, Sect. 20, 24 July 1956. This species has been reported from pine forests in southern Michigan previously (Smith, **1951**) and R. F. Cain has found it under similar jack-pine forest in Ontario.

The collections reported above extend the range and habitat type for three species of subterranean fungi. They also suggest that these fungi are not rare, but rather uncollected (like the Tuberales) because of their hidden position.

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LITERATURE CITED

Bessey, E. A. 1950. Morphology and taxonomy of the fungi. The Blakiston Co., Philadelphia, Pa.

Dodge, C. W. 1929. The higher Plectascales. Ann. Mycol. **27**: 145–184.

Fries, E. M. 1825. Systema Orbis Vegetabilis, pp. 134–135.

——. 1829. Systema Mycologicum **3**: 58–59.

Lanjouw, J., et al. 1956. International Code of Botanical Nomenclature. Regnum Vegetabile Vol. 8.

Mains, E. B. 1957. Species of *Cordyceps* parasitic on *Elaphomyces*. Bull. Torr. Bot. Club **84**: 243–251.

Persoon, C. H. 1801. Synopsis methodica Fungorum. Göttingen.

Smith, A. H. 1951. Puffballs and their allies in Michigan. University of Michigan Press. Ann Arbor, Michigan.

FIG. 1 (top). Shallow digging by deer indicating presence of *Elaphomyces* and *Rhizopogon*. Trowel gives scale. FIG. 2 (middle). Open jack pine forest—habitat of subterranean fungi. FIG. 3 (bottom). Photomicrograph (× 133) showing asci of *Elaphomyces anthracinus*.

MODIFICATIONS OF THE CELLOPHANE CULTURE TECHNIQUE FOR PHOTOGRAPHING AND PRESERVING REFERENCE COLONIES OF MICROORGANISMS [1]

W. T. Kondo, S. O. Graham, and C. G. Shaw [2]

(with 1 figure)

In recent years, many workers have used non-moisture-proof cellophane as a surface atop solid media on which to culture both bacteria and fungi in a manner suitable for preservation of the colonies. Fleming and Smith [3] first suggested the technique for preservation of colonies *per se* and for production of cultures that could be mounted for microscopic observation. Jones [4, 5] modified the technique to some extent and used it to preserve specific isolates for the herbarium (particularly to accompany type specimens), for classroom material, and for exchange between workers. This paper reports several modifications of the basic technique which increase its usefulness.

NOTES ON THE BASIC TECHNIQUE

There are advantages and disadvantages of close contact between the solid medium and the cellophane discs.[6] In the study of colony characteristics it is mandatory that complete contact be obtained; otherwise, the cellophane is wrinkled and not flush with the medium, and the colony grows unevenly, being sparse in the disjunct areas. Although Jones [4, 5] advocates sparse growth as the best for microscopic mounts, it obviously

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[3] Fleming, Alexander, and George Smith. 1944. Some methods for study of moulds. Brit. Mycol. Soc. Trans. **27**: 13–19.

[4] Jones, Walter. 1950. Preparation of permanent mounts of fungus colonies on cellophane. Phytopathology **40**: 967. (Abstr.).

[5] Jones, Walter. 1956. Permanent mounts of fungus cultures on cellophane. Phytopathology **46**: 131–132.

[6] Cellophane distorts and wrinkles badly unless evenly and completely saturated with water.

is not suitable for determining colony characteristics and pigmentation.

Fleming and Smith [5] reduced wrinkling by sterilizing their cellophane discs between layers of moist blotting paper. However, even then some wrinkling occurred and it was necessary to straighten the discs after they had absorbed moisture from the medium. Carmichael [7] used small discs (15–18 mm) in his work with no apparent difficulty from wrinkling. However, these discs were too small for large-colony growth characteristics to become manifest.

In our work with larger cellophane discs (7 cm), we also experienced a considerable wrinkling problem, which was overcome by the following procedure:

1. Alternate 7 cm cellophane discs between filter papers and place in a deep petri dish.
2. Autoclave dry at 15 lb for 20 minutes.
3. With materials at ambient temperatures, use sterile forceps to dip individual sterile cellophane discs into sterile distilled water and then arrange on the surface of suitable 2.5% agar [8] in a poured plate.
4. If bubbles occur, raise the edge of cellophane disc with the forceps and force the bubbles out by rolling the disc back to the agar surface slowly.
5. Allow the prepared plates to incubate 48 hours. This ensures freedom from contaminations and provides time for excess moisture to be absorbed by the medium.

Inoculating a too moist cellophane surface with a spore suspension will allow dispersion of the inoculum and irregular colonies will develop. In our colony comparison studies we have found it advantageous to use biscuits of inoculum cut from donor plates rather than to use inoculum suspensions. The biscuit is removed as soon as growth of the colony is well started.

Tests were conducted to compare the relative rates of growth of different organisms with and without a disc of cellophane between the inoculum and the medium. There were no apparent critical differences between colonies in relative rates of growth, colony surface characteristics, and pigment development in any of the organisms tested. In most cases where cellophane discs were used, colony growth was restricted to

[7] Carmichael, J. W. 1956. The cellophane technique for studying morphology and hyphal fusion in fungi. Mycologia **48**: 450–453.

[8] 2.5% agar was used instead of the conventional 2% to allow uptake of excess moisture from the cellophane disc.

the surface of the disc. Among those genera that did grow into the medium, *Alternaria, Stemphylium,* and *Cephalosporium* penetrated the cellophane, whereas *Colletotrichum, Gloeosporium* and *Glomerella,* and the various wood-rotting fungi digested it.

PRESERVATION OF COLONIES AS HERBARIUM SPECIMENS

Jones [4, 5] has described methods for preparing cultures on cellophane for herbarium purposes and has emphasized their advantages over dried agar cultures.

The culture holder illustrated in FIG. 1A has been designed for filing cellophane cultures in the Mycological Herbarium, Department of Plant Pathology, State College of Washington (WSP).[9] The front of the holder measures 155 × 94 mm. The back flap, which folds under, measures 100 × 94 mm. The lower half of the front serves as the label for the culture. Space is provided for the essential data pertaining both to the original specimen from which the culture was obtained and the culture itself. "A. S. No." refers to the Accession number of the specimen (i.e. culture).

An opening (55 × 55 mm) is cut through the upper portion of the front and back flap. This size has been found most satisfactory for cultures grown on cellophane discs approximately 80 mm in diameter. The cellophane disc bearing the culture is placed between the front and back flap and stapled in position. The overall size of the culture holder is such (155 × 94 mm) that, with a culture in place, the holder can readily be slipped into "cover-size" (width 97 mm) Crystal Mounts.[10] The Crystal Mount protects the culture and provides a ready means of mounting the holders on herbarium sheets.

The desirability of depositing dried cultures of microorganisms in herbaria cannot be overemphasized. If it is necessary to make isolations and to obtain cultures in order to identify a fungus or a bacterium, it is obvious that the material originally collected does not constitute a suitable specimen of the organism. In such instances the preservation of dried cultures should be axiomatic if anything at all is to be preserved as confirmation of the occurrence of the microorganism on a particular substrate or in a particular geographical area. If new species are involved, dried subcultures grown on cellophane are the best possible type.

[9] Lanjouw, J., and Stafleu, F. A. Index Herbariorum. Part I. The Herbaria of the World. Ed. 3. 224 pp. Intern. Bur. Pl. Tax. and Nomencl., Intern. Assoc. Pl. Tax. Utrecht. 1956.

[10] Crystal Mount is the trade name for cellulose-acetate tubing with an adhesive strip attached. It is sold under the Honor Built trademark by H. E. Harris & Co., Boston 17, Massachusetts.

Even though living cultures can be maintained, a subculture derived from the original isolate preserved as a dried specimen serves several useful purposes. By comparing the dried with the living cultures, one

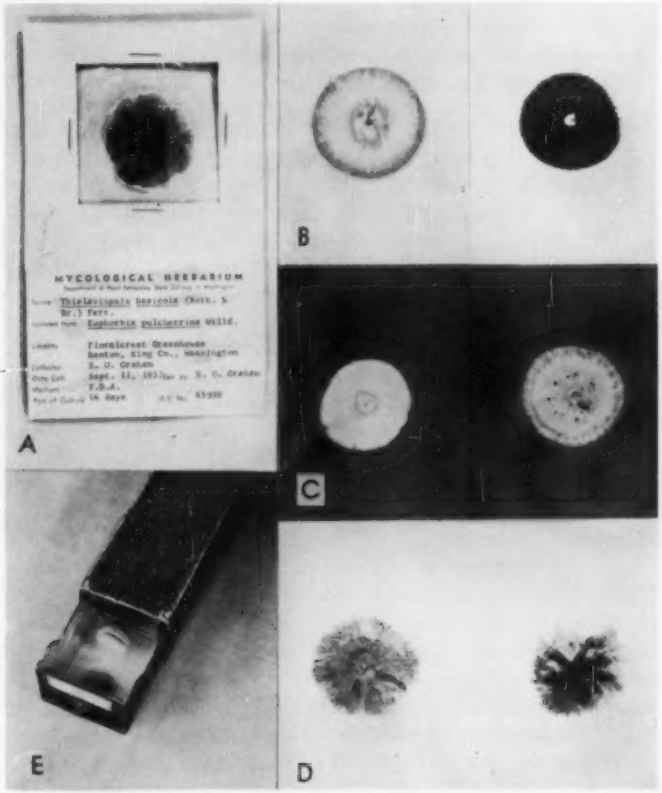

FIG. 1. A. Typical cellophane culture holder used in the Mycological Herbarium, Department of Plant Pathology, State College of Washington. B. *Helminthosporium inconspicuum* Cke. and Ell., colonies mounted on a glass plate. Left, upper surface of a colony; right, lower surface, showing pigmentation. C. *Fusarium moniliforme* Sheldon, colonies illustrating the contrast obtained by employing a dark background. Note evidence of saltation which would be shrouded if the culture had been photographed through an agar medium. Left, upper surface of a colony; right, lower surface of a colony. D. *Thielaviopsis basicola* (Berk. and Br.) Ferr., colonies illustrating effect obtained with transmitted light. Left, upper surface of a colony; right, lower surface. E. 3″ × 5″ card-file box converted for storage of reference colonies on glass.

can easily ascertain if the living culture has been displaced by a contaminant or if its morphology has changed markedly as a result of saltation or mutation. If the living culture is inadvertently lost, the dried culture constitutes a permanent record.

PRESERVATION OF REFERENCE COLONIES FOR GROWTH AND PIGMENTATION RECORDS AND PHOTOGRAPHY

A colony grown on its cellophane carrier, mounted on a labeled holder, and placed in a protective cover is satisfactory for normal herbarium or classroom application. However, with such treatment, uneven shrinkage during drying of the cellophane carrier distorts the surface. This distortion makes it difficult to measure accurately critical differences between reference and subsequent colonies. We need to preserve colonies in typical habit for size, sectoring, and pigmentation comparisons from year to year.

Mounting the cellophane carrier onto a rigid vehicle (glass or rigid plastic) has alleviated the above difficulty and has increased the usefulness of reference colonies preserved on cellophane. As FIGS. 1B and 1C show, a transparent vehicle such as the glass plates shown preserves the colony for accurate reference measurements, and greatly enhances photographing both sides of a colony. There is no petri plate or nutrient medium to obstruct viewing and photographing pigmentation or undisclosed sectoring phenomena. The pigmentation is not only intense and well defined, it is permanent as a reference record where such characters are important, e.g., in the identification of species of *Penicillium, Aspergillus, Fusarium,* etc. Also, in photographing a colony mounted on a transparent plate, one can effectively manipulate the background to accentuate growth characteristics that are too shrouded by the medium to capture. (FIG. 1C illustrates manipulation with a dark background while FIG. 1D illustrates the effect one can obtain with transmitted light.) The reflecting concave surfaces of the culture media and the edges of petri plates which usually plague one during photographing are eliminated. Furthermore, by mounting on uniform-size plates, one has a versatile tool with which to compose mosaics for equivalent comparisons that are free from the optical illusions often caused by petri plates.

In our work we prefer small glass plates as the rigid vehicle for several reasons. They are readily available in appropriate 9×12 cm size from discarded Kodak M plates, which are very thin and relatively easy to file. Glass does not scratch as easily as plastic, and the uniform size allows ready assembly of colonies into diverse mosaics.

An appropriate adhesive to cement the cellophane disc to the plate without defacing the colony is a limiting requisite, and one must be cautious in manipulation of the disc during mounting.

Most dehydrating agents not only wrinkle and cause easy fracture of the cellophane, but also are absorbed by the colonies and mar their natural appearances. Pure glycerine, Shear's mounting fluid, lactophenol and most other hygroscopic mounting media make excellent solutions with which to arrange the cellophane but were rapidly absorbed by colonies and thus defaced them, as did the anhydrous adhesives.

The most satisfactory medium tested was one containing gelatin to stick the cellophane, glycerine as a hygroscopic agent to prevent sloughing and splitting of the cellophane during drying and in storage, and phenol as a preservative. The most suitable formulation of this medium follows:

Glycerine	3.0 ml
Gelatin	3.0 g
Phenol	0.2 g
Water	100.0 ml

On occasion it was found necessary to increase the glycerine content of the medium to prevent splitting of the cellophane during drying of certain colonies, e.g., those of the genera *Stemphylium*, *Alternaria* and *Cephalosporium*. Caution should be taken in this regard, as a colony can be ruined for reference if the glycerine is in great enough concentration to soak into it and mar its surface.

In mounting the cellophane carrier onto the glass plate, the procedure listed below offered the easiest manipulation:

1. Place a generous amount (about 0.5–1.0 ml) of mounting fluid on a clean glass plate.
2. With a pair of forceps gently peel a cellophane carrier and its colony from a petri plate, remove any adhering culture medium with a scalpel, and float the carrier on the medium.
3. If bubbles are present under the cellophane, lift the edge of the cellophane disc and roll it down slowly to displace them.
4. With a soft camel's-hair brush and a piece of filter paper as a blotting agent, center the colony on the plate and express the excess mounting fluid from beneath the carrier.
5. Allow the plate to dry slowly. Some wrinkling may occur before the mounting fluid has completely dried. The main concern is to remove the trapped air from beneath the cellophane because these loci will not fuse to the glass on drying and will cause artifacts.

This air removal is particularly important in areas beneath the colony itself.

Storage of reference colonies on glass should not pose a great problem. Protective cases are easily made of discarded wooden microscope-slide boxes and a 3 × 5″ card-file box. Disassemble the wooden microscope-slide box and glue the sides to the sides of the card-file box. The result is an excellent protective file in which colonies can be catalogued easily (FIG. 1E).

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NEW AND INTERESTING SPECIES OF BASIDIOMYCETES. VI [1]

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(WITH 19 FIGURES)

LACCARIA LACCATA var. PROXIMA (Boudier, Bull. Soc. Myc. Fr. **28**: 91. 1881); R. Maire, Bull. Soc. Myc. Fr. **24**: lv. 1908 FIG. 1

This variety, which, when the stirps *Laccata* is finally monographed, may easily become an autonomous species (as had been proposed by Boudier), has been described macroscopically by Boudier, Lange, Møller and others. However, the anatomical description has remained rather incomplete. Consequently, my notes are given below:

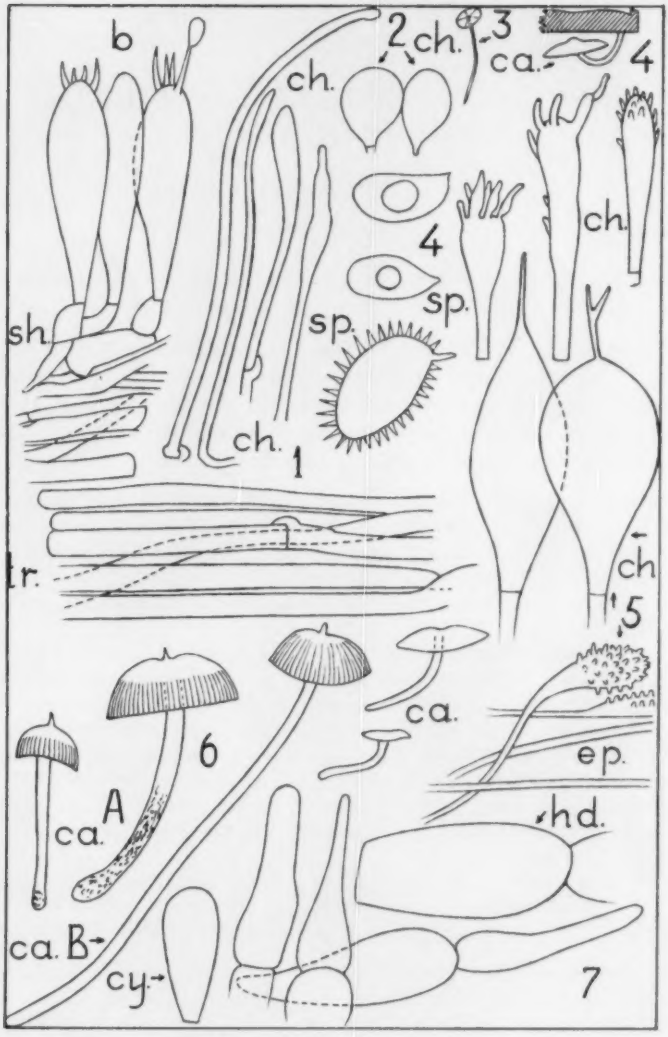
Spores: (10–)11–12.8 × 7.5–9.5 μ, varying from as broad as 11.3 × 9 μ to as narrow as 12 × 7.5 μ, definitely narrower on the average than any other form of the *Laccata*-group, hyaline, inamyloid, spines narrowly conical or more rarely thorn-shaped, (1.2–)1.5(–2)μ long (spore measurements *with* ornamentation), covering the spore completely without a plage.

Hymenium: Basidia 30–38 × 8.5–11.5 μ, narrowly clavate, 4-spored. Cystidia none. Cheilocystidia scattered among basidia and basidioles on edges of lamellae and nearby, of two types, (1) long-filamentous and reminding one of the cheilocystidia of *Tricholoma albobrunneum* and *T. stans*, 2–2.5 μ in diameter, hyaline, often projecting, and (2) short-filamentous and often with a claviculate head which may or may not taper into a primary and a secondary mucro, hyaline, 15–36 (length measured from a basal knee or septum, whichever is higher) × 2.5–3 μ (head 3.5–4.3 μ).

Hyphae with clamp connections. Hymenophoral trama regular, consisting of parallel hyphae, eventually slightly interwoven but still subparallel, filamentous, hyaline. Epicuticular layer little differentiated.

These specimens were collected (and recorded) by J. Favre, September 14, 1938, in the Haut-marais des Rousses, 1050 m, French Jura (MICH).

[1] Previous contributions under this title were published in Mycologia **37**: 425–439. 1945; II: Pap. Mich. Acad. **32**: 103–150. 1948; III: Sydowia **4**: 130–157. 1950; IV: Mycologia **47**: 763–777. 1955; V: Sydowia **11**: 141–272. 1957.



FIGS. 1-7.

FIGS. 1-7. FIG. 1. *Laccaria laccata* var. *proxima* (Boud.) Maire. b. basidia, one with immature spore; sh. subhymenium; tr. hymenophoral trama; ch. cheilocystidia; sp. spore. FIG. 2. *Laccaria laccata* var. (Mexico). ch. cheilocystidia.

Although some authors measure the spores without the ornamentations, unfortunately often without saying so, it seems improbable that the small-spored form, described under the same name by Möller from the Faeroes, could possibly have similar spore measurements, inasmuch as it is said to be shortly and densely spinulose.

It should be possible to find out whether Boudier's original material or authentic collections, most probably at the Herbarium of the Muséum d'Histoire Naturelle in Paris, agree with Favre's concept and the above redescription, or with the description given by Möller. Furthermore, there is a variety (according to Kühner & Romagnesi) which differs in lilac lamellae and amethyst basal tomentum, and which these authors call *Laccaria proxima* var. *bicolor* Maire. There are, according to my own experience, two forms in this group which have amethyst-colored basal mycelium. One of these was described by me (Ann. Mycol. **41**: 17. 1943) as *Laccaria laccata* var. *rosella* (Batsch.) Sing.; it differs clearly in more globose spores and perhaps a less lilac tone of the lamellae. I believe that this is indeed the species or form depicted by Batsch. Furthermore, there is another species with amethyst-colored base, a form which I have found only in America (Florida, *Singer F 1672a*, FH, F; and Mexico, *Singer M 1581*). The Mexican collection, at least, differs from the European one in having markedly vesiculose cheilocystidia (FIG. 2) and flesh-color lamellae, while the Florida form has more lilac lamellae (between lilac and flesh color) but the cheilocystidia have not been restudied recently. It is not possible at present to coordinate all these data with published names. If Favre's species is the true *L. proxima*, it should become unavoidable to consider it as a species independent of *L. laccata*.

Since *L. ohiensis*, according to my own investigations, occurs in Sweden, and *L. tetraspora* in England, it becomes necessary to revise the laccarias of Europe, and, first of all, designate a type form to represent the European *laccata*. Obviously, this would have to be a specimen from Sweden, with small spores and short (less than 1 μ) spinules.

My complete redescriptions of various forms of this stirps (*L. ohiensis, L. tetraspora, L. laccata* var. *roseola, L. laccata* var. from Mexico,

FIG. 3. *Hemimycena subtropicalis* Sing. carpophore. FIG. 4. *Mycena austinii* (Peck) Kühner. ca. carpophore, growing from underside of wood; sp. spores; ch. cheilocystidia. FIG. 5. *Mycena austroalcalina* Sing. ch. cheilocystidia; ca. carpophore; ep. hyphae of the epicutis of the pileus, with hd. hypodermial hypha. FIG. 6. *Mycena* (?) *humilis* Rick. caA. carpophores from dicotyledonous log; caB. carpophore from palm trunk. FIG. 7. *Mycena heroica* Sing. cy. cystidia. (FIG. 1, sp, 4, sp, × 1780; all other microscopical elements × 890; all macroscopical drawings marked ca. × 1.)

and now *L. laccata* var. *proxima* from France) are not meant to settle the nomenclature of these forms once and for all, but rather to show the polymorphic character of the stirps and the various constant forms that exist and have been thrown together by many authors in the past.

A form extremely close to *L. laccata* and formerly always determined by me as such is *L. laccata* var. *montana* as described by Möller. This same form is also common in Greenland, e.g., Godtbaal, Aug.–Sept. 1944, coll. *Haomi Jackson,* comm. Snell, det. Singer (FH). It differs from the normal form merely in the darker pileus (which may not be a constant character) and the smaller average size of the pileus and shorter stipe.

Gerronema subpallidum Sing. spec. nov.

Pileo pallido, interdum obscure brunneo in disco, haud fibrilloso, nec striato nec sulcato, globoso dein convexo, centro dein applanato vel depresso, haud papillato, 12–14 mm lato. Lamellis albis, arcuatis, decurrentibus, distantibus. Stipite albo, 10–14 × 2–4 mm. Sporis 6.8–7.5 × 3.8–4.8 μ; cystidiis nullis; hyphis defibulatis.

Pileus pallid, in one specimen of the type collection with a deep brown disc, otherwise uniformly pallid, not innately radially fibrillose, glabrous all over, not transparently striate or sulcate, but margin somewhat coarsely short-grooved or plicate, not viscid, not quite opaque when dried, globose, then convex, with applanate to depressed center, in dried specimens with deeply depressed center, 12–14 mm broad.

Lamellate white, arcuate, distant, deeply decurrent.

Stipe white, glabrous, smooth, not insititious, cylindric, 10–14 × 2–4 mm.

Context white; odor none.

Spores 6.8–7.5 × 3.8–4.8 μ, hyaline, smooth, ellipsoid, inamyloid.

Hymenium: Basidia 26.5–30 × 6–7 μ, very irregular, without a basal septum, or with a septum, clavate, often 1- or 2-spored, often not forming a continuous hymenium and frequently interrupted by cystidioles or hyphae which break through the hymenial layer. Cystidiole-like elements particularly numerous near the edge, often "empty." True cystidia and cheilocystidia not differentiated.

Hyphae without clamp connections, inamyloid; flesh of pileus consisting of irregularly interwoven, often thick-walled elements; subhymenium moderately distinct, of small elements. Hymenophoral trama not regular, with narrow and broad elongated elements, the broad ones and some of the narrow ones more or less thick-walled, others quite thin-walled, some hyphae involute-spiralling, but not or scarcely gelatinized; septa distant from each other.

Covering layer of pileus: Epicutis hyaline (where pileus pallid), its hyphae sometimes somewhat uneven but not ramose or diverticulate,

thin-filamentose, repent-appressed; hypodermium hyaline (in pallid zone), consisting of hyphae broader than those of the epicutis, somewhat interwoven, forming a cutis.

On dead dicotyledonous branches in subtropical forest, summer.

Argentina: Prov. Tucumán, Rio de los Sosas, 1–III–1956, *Singer T 2248*, TYPE (LIL).

This is intermediate between *G. depauperatum* and *G. melanomphax*. It is the only *Gerronema* which suggests a *Marasmius* when fresh; it resembles particularly *M. podocarpi* Sing.

Gerronema angustifolium Sing. spec. nov.

A *G. subpallido* differt carpophoris tenuioribus minoribus, pileo atro-fibrilloso, lamellis paulum angustoribus, sporis longioribus constanterque binis.

Pileus hyaline, with blackish fibrils (which are very small, applicate and radially arranged), convex, umbilicate, eventually applanate-umbilicate, with sulcate margin, 5–13 mm broad.

Lamellae white, very distant, narrow to very narrow and almost venose, 0.4–1.8 mm broad, irregularly intermixed, 8–13 through-lamellae present, arcuate-descendant, decurrent to deeply decurrent.

Stipe hyaline, fibrillose like the pileus, but in lower portion glabrous and without dark fibrils, equal, rarely with a small indistinct bulbulous base, 7–25 × 1–2 mm.

Context pallid, thin, unchanging, inodorous.

Spores 8–8.5 × 4.8–5.5 μ, hyaline, inamyloid, smooth, ellipsoid.

Hymenium: Basidia 30–42 × 4.8–6.8 μ, long-clavate (almost 5 times as long as broad), constantly 2-spored. Cystidia none, but some basidiole-like bodies remaining sterile especially at and near edges.

Hyphae without clamp connections, inamyloid, irregular-subintermixed in the hymenophoral trama but in the lateral stratum with bunches of parallel hyphae running in a generally axial direction, hyphae thin-walled, few very slightly thick-walled; hyphae of cuticular region radially arranged with thin to rather thin walls, with intracellular umber pigment, all smooth; dermatocystidia none.

On trunks, fallen branches and twigs of frondose trees in tropical hammock vegetation, fruiting towards the end of the rainy season.

U.S.A.: Florida, Dade County, Brickell's Hammock (Miami), 25–X–1942, *R. Singer F 1285*, TYPE (FH).

This species is very close to the preceding one and differs in the characters enumerated in the Latin diagnosis.

The following species (aside from the above) enter the genus *Gerronema: G. elasticum* Sing.; *G. melanomphax* Sing.; *G. depauperatum*

Sing. Aside from these three species (as described originally: New Genera of Fungi V, Mycologia **43**: 598–604. 1951), we refer to this genus also the following species formerly referred to *Marasmiellus* (as sect. *Xanthophylli*):

G. stuckertii (Speg.) Sing. comb. nov.
Omphalia stuckertii Speg., An. Mus. Nac. Buenos Aires **8**: 49. 1902.
Marasmiellus stuckertii (Speg.) Sing., Lilloa **23**: 191. 1950.
Omphalia bruchii Speg., Bol. Acad. Nac. Cienc. Nat. Cordoba **28**: 286. 1926.

G. subchrysophyllum (Murr.) Sing., Sydowia **11**: 357. 1957.
Omphalina subchrysophylla Murr., Jour. Fla. Acad. Sci. **7**: 112. 1944.
Omphalia subchrysophylla (Murr.) Murr., Jour. Fla. Acad. Sci. **7**: 127. 1944.
Marasmiellus subchrysophyllus (Murr.) Sing., Lilloa **22**: 299. 1949.

G. icterinum (Sing.) Sing., Sydowia **9**: 373. 1955 ex Sing. comb. nov.
Marasmiellus icterinus Sing., Sydowia **2**: 30. 1948.
Omphalina euomphalus var. *lutea* Dennis, Kew Bull. **1952**: 496. 1952.

One species which had been referred to *Armillariella* by the present author in 1951 is better placed in the genus *Gerronema*, near the preceding species but differing in yellow spore print and lack of clamp connections:

G. chrysophyllum (Fr.) Sing. comb. nov.
Agaricus chrysophyllus Fr., Syst. Mycol. **1**: 167. 1821.

The following species had been accommodated temporarily in *Phaeomycena*, but belong in *Gerronema* although they differ in pigments; the second is not uncommon from Cuba south to Argentina (Prov. of Salta).

G. macrosporum (Sing.) Sing. comb. nov.
Phaeomycena macrospora Sing. in Sing. & Digilio, Lilloa **25**: 175. 1951.

G. cyathiforme (Berk. & Curt.) Sing. comb. nov.
Marasmius cyathiformis Berk. & Curt., Journ. Linn. Soc. Bot. **10**: 295. 1869.
Omphalina cyathiformis (Berk. & Curt.) Dennis, Kew Bull. **1952**: 494. 1952.

Furthermore, I suspect that *Clitocybe xanthophylla* Bres. belongs here.

Hemimycena subtropicalis Sing. spec. nov. FIG. 3

Species stirpi *H. crispulae* et *H. pseudocrispulae* (Kühner) Sing. paraffinis a qua differt pileo stipiteque minoribus et forma elementorum epicutis pilei nec non habitatione.

Pileus white, convex, obtuse, not umbilicate, glabrous and smooth, membranous, 1.5–1.8 mm.

Lamellae white, 3–5 in number, venose or very narrow, not reaching the extreme margin of the pileus, extremely distant, subdecurrent.

Stipe white, glabrous above, pilose below, filiform, insititious, 9 × 0.1 mm.

Context inodorous, extremely thin.

Spores 9.3–12 × 2.7–4.5 μ, hyaline, thin-walled, elongate, smooth, inamyloid.

Basidia (16–)20–21 × 5.3–6.7 μ, 2–3–4-spored; basidioles fusoid. Cheilocystidia and cystidia none.

Hyphae with clamp connections; in the flesh of the apex of the stipe voluminous, forming a tissue of vertical parallel elements reaching nearly the surface of the pileus; other hyphae of pileus radially arranged, all hyphae inamyloid.

Covering layer of pileus an epicutis consisting of some horizontal appressed hyphae and some characteristic clavate-ventricose or vesiculose-clavate elements, the latter often in fascicles, but also frequently solitary, and all these elements of the epicutis distinctly diverticulate; the broad elements well differentiated, 10–24 × 8–14 μ, with broadly rounded tip. Covering layer of stipe with only occasional diverticulate elements, but, especially in the lower portion, with numerous pilose cells about 40 μ long, narrowly subulate, and strictly acute.

On petioles and fruits of a dicotyledonous tree in the subtropical forest (lower zone), fruiting in summer.

Argentina: Prov. Tucumán, Parque Aconquija, 17–II–1955, *Singer T 2185*, TYPE (LIL).

This species is very close to *H. pseudocrispula* (Kühner) Sing., from which it differs in smaller size, different shape of the epicuticular bodies, and different habitat (leaf petioles of evergreen dicotyledonous tree in subtropical vegetation instead of grasses and herbs).

It belongs, together with related species, to a group of mycenoid agarics which have been described as *Omphalia*, but were transferred to *Mycena* by some mycologists who have, in the past, used a very broad concept of the latter genus. These species were classified in the subgenus (or genus) *Hemimycena* Sing. Lately, Singer combined this subgenus or genus with the genus *Marasmiellus*, while Kühner & Romagnesi combined it with *Delicatula*. As has been pointed out in a

recent paper, the species related to *M. lactea, M. crispula,* etc., should now be separated from *Delicatula* as well as *Marasmiellus,* to be treated as autonomous genus *Hemimycena* (whose type species is *H. lactea*).

Mycena aconquijensis Sing. spec. nov.

Pileo albo, convexo dein applanato, usque ad 30 mm lato. Lamellis albis, angustis, confertis vel subconfertis, adnatis vel subdecurrentibus. Stipite albo, sicco, 75 × 2 mm. Sporis 4.7–5.3 × 2.3–2.8 μ, inamyloideis vel subinamyloideis, levibus; basidiis 4-sporis; cystidiis nullis; cheilocystidiis filamentosis; tramate amyloideo; epicute subpelliculosa, pro ratione dense, hyphis haud diverticulatis.

Pileus white, not viscid or at the most almost subviscid, glabrous, convex, then flat, subumbonate, submembranaceous, often broken deeply in the center, in older caps the depression sometimes connecting with the hollow of the stipe, center becoming very pale cinnamon and sometimes with sordid salmon-colored blotches or patches in very old material, up to 30 mm broad.

Lamellae white, slightly crisped to slightly intervenose, narrow, close or subclose, adnate to subdecurrent, in old caps sometimes flesh-colored or salmon-blotched.

Stipe white, tending to gray near base, eventually somewhat flesh-color-blotched, glabrous or subglabrous, not viscid, hollow, equal, 75 × 2 mm.

Context white; odor of crustaceans.

Spores 4.7–5.3 × 2.3–2.8 μ, smooth, either practically inamyloid or very slightly amyloid (but there are spore-wall fragments remaining among the basidia which are distinctly amyloid).

Hymenium: Basidia 4-spored, inamyloid. Cystidia none. Cheilocystidia 1.5 μ thick, numerous, filamentous, often with short irregular lateral branchlets or distantly nodulose, hyaline.

Hyphae of the regular gill trama amyloid, as are all other hyphae of the carpophore.

Covering layer of pileus: Epicutis consisting of a pellicle of hyaline hyphae which are indistinctly incrusted, very thin, relatively dense and very slightly gelatinized. Outer layer of stipe rind not gelatinized, consisting of appressed (only rarely slightly projecting) hyphal ends.

Fasciculate-cespitose in large numbers on dead trunks of dicot trees in subtropical forest at about 900–1000 m alt.

Argentina: Prov. Tucumán, Taficillo (East slope), 23–II–1955, *Singer T 2205,* TYPE (LIL).

This species when fresh looked like one of the *Marasmii* near *M. niveus* although plainly mycenoid. It reminds one somewhat of *Mycena insignis* Smith, but spores and habitat are quite different.

MYCENA AUSTINII (Peck) Kühner, Le Genre Mycena, p. 394. 1938. FIG. 4

Agaricus austini Peck, Ann. Rep. N. Y. State Mus. **28**: 48. 1876.
Omphalia austini (Peck) Sacc., Syll. **5**: 336. 1887.
Omphalopsis austini (Peck) Murr., N. Am. Fl. **9**: 312. 1916.

Pileus white to sordid gray, deep sulcate-striate, viscid when wet, glabrous, about 12 mm broad, convex with umbo, on drying becoming convex-umbilicate with deep umbilicus.

Lamellae white or grayish white, narrow to rather broad (most frequently about 2 mm broad), subdistant to distant (15–17 through lamellae), adnate-decurrent (more decurrent on drying), sickle-shaped.

Stipe colored like the pileus and lamellae, somewhat viscid when wet, smooth and glabrous, often curved, equal, hollow, 13–14 × 1.5 mm; base white-strigose and slightly thickened.

Context pallid, thin, inodorous, slightly tough-elastic.

Spores 7.5–8.5 × 3.8–4.5 μ, ellipsoid to oblong, often somewhat applanate on the inner side, smooth, hyaline, amyloid.

Hymenium: Basidia 21 × 6–6.5 μ, 4-spored. Cystidia few, generally like cheilocystidia or somewhat more branched. Cheilocystidia 30–43 × 6.5–7 μ, with apical erect or oblique finger-like appendages (at least 2, more often 4, but frequently up to 10), appendages fusoid, filiform, conical and acute, etc., sometimes spreading beyond the diameter of the main portion (i.e. to 9–14 μ), 3.5–11.5 μ long, main body sometimes forked or with branching excrescences.

Hyphae: all with clamp connections, some more or less amyloid, those of subhymenium and covering layers gelatinized, also edge of lamellae somewhat gelatinized, in epicutis often somewhat ring-like-spirally incrusted, otherwise not distinctly diverticulate, filamentous; hymenophoral trama regular, becoming irregularly interwoven in age.

Gregarious (not fasciulate-cespitose) on bark of a dead trunk of *Thuja*, fruiting in (August and) September.

U. S. A.: N. Y., Newcomb Co., Huntington Forest, Wolf Lake, September 12, *Singer 153* (FH).

This collection was determined by A. H. Smith, who has studied the type (see N. Am. Species of Mycena, p. 408. 1947). The species seems to be rare, and consequently a full description of an apparently typical collection is here supplied.

The type was collected on spruce (*Picea*). The species occurs apparently on various conifers. It seems to differ from *M. glutinosa* Beardslee in slightly less strong amyloid reaction, in habit (not in dense cespitose masses) and habitat, and in slightly smaller measurements.

Mycena austroalcalina Sing. spec. nov. FIG. 5

Pileo griseo, viscido, convexo, glabro, 8–15 mm lato. Lamellis albis, liberis. Stipite albido, basin versus sordide griseolo, levi vel sublevi, paulum fibrilloso ad basin, haud viscido, 12–25 × 1–1.8 mm. Odore forti alcalino. Sporis inamyloideis. Hyphis amyloideis. Cheilocystidiis tenui-appendiculatis.

Pileus gray, hygrophanous, fading to pale gray, viscid, in dry weather quite dry, dried white and striped and smooth, fresh sulcate, convex, then applanate, or convex with depressed center, 8–15 mm broad.

Lamellae white, moderately close to subclose, ventricose, moderately broad to rather broad, free or subfree. Spore print pure white.

Stipe whitish, very slightly sordid grayish at base, smooth to (s.l.) scarcely longitudinally innately fibrillose-striate, glabrous, hollow, equal, not insititious, but with relatively little basal fibrillosity, not viscid when collected, 12–25 × 1–1.8 mm broad.

Context whitish, unchanging, very thin; odor distinctly and strongly of chlorine as in *M. alcalina*.

Spores 8.2–8.8 × 5.7–6.4 μ, ellipsoid, smooth, inamyloid.

Hymenium: Basidia 24–27 × 6.7–8.8 μ, 4-spored, some 2-spored. Cystidia none. Cheilocystidia rising directly from the hyphae of the trama, "empty," vesiculose to ventricose, 29–47 × 10.7–21.5 μ, with thin apical appendages in most (appendages often long: up to 13 μ) and forked (measurements of length do not include appendages), entirely hyaline.

Hyphae: all with clamp connections, distinctly amyloid, many inflated, radially arranged in pileus, regular in hymenophoral trama (and there particularly voluminous), gelatinized in epicutis of pileus and the outermost hyphae there repent and diverticulate (strongly so) and slightly thicker than the gelatinized ones below. Surface of stipe not visibly gelatinized, with occasional dermatocystidia which are very similar to the cheilocystidia.

On dicotyledonous trunk in subtropical forest, summer-fruiting.

Argentina: Prov. Tucumán, Rio de los Sosas, 4–II–1955, *Singer T 2122*, TYPE (LIL).

This species is perhaps close to *M. margarita* Murr.

MYCENA (?) HUMILIS Rick, Lilloa **2**: 287. 1938. FIG. 6

Pileus fuscous, between *Conga* and *Montella* (M & P 8–H/J–11), later between *Malay* and *Clove* (M & P 15–C–11/12), the extreme margin sometimes white, or the entire pileus no more than grayish white, with a concolorous or almost black characteristically slender and acute strongly projecting papilla, slightly to strongly radially innately fibrillose, not viscid, more or less hygrophanous, convex to convex-campanulate, later convex with applanate-papillate center or eventually with

depressed center, 10–50 mm broad, margin sulcate over $\frac{1}{3}$ to $\frac{2}{3}$ of radius.

Lamellae white, more rarely with a slight fuscous shade in the inner-upper half, subdistant to distant, broad, at first ascendant, soon becoming horizontal, more or less ventricose, deeply sinuate and narrowly adnexed. Spore print pure white.

Stipe with white or hyaline or silvery to cinereous-white ground color and above with innate longitudinal white fibrils, substriate, below with appressed umber-gray fibrils in many individuals or populations although frequently whole populations without any umber, dry, subequal, at times slightly thickened at base, rarely tapering downward or upward, 30–90 × 3–5 mm; veil none;[2] basal tomentum white.

Context white, unchanging, relatively firm; odor weakly but distinctly of *M. alcalina* (of chlorine).

Spores 8.5–11 × 4.7–5.3 μ, smooth, hyaline.

Hymenium: Basidia 27–28 × 7 μ, few 2-spored, mostly 4-spored. Cystidia numerous on the sides of the lamellae, 62–73 × 11.3–14.7 μ, ampullaceous, varying from obtuse to strictly acute, hyaline, entire, often with ellipsoid enclosures, rarely granular-incrusted; those at edge (cheilocystidia) similar although slightly more variable.

Hyphae: with numerous clamp connections, but in some individuals, especially those that are substerile, with few or no clamps, amyloid. Hymenophoral trama regular, with voluminous hyphae and filamentous ones.

Covering layers of repent parallel hyphae both on pileus and stipe and the epicutis of the former without a gelatinous layer, diverticules scattered in center, crowded toward the margin, of ordinary size or taking the shape of long erect hyphal ends, especially near the margin where they are cylindric, ventricose, ampullaceous or clavate, mostly clavate (in center mostly cylindric), up to 3 μ long (if of the short type), or up to 8 μ long if cystidioid. Hypodermium of parallel voluminous hyphae forming a cutis and differing from the hyphae of the context of the pileus in having a more regular structure.

Growing on wood of mono- and dictyledonous trees, on palms sometimes climbing up to 4 m above the ground, but also on rotting wood of Myrtaceae, etc., at the ground level, fruiting from spring until fall. Common.

Brazil: Rio Grande do Sul, TYPE (Collegio Anchieta)—Villa Manresa near Porto Alegre, 29–X–1951, *Singer B 12* (LIL).—Argentina: Prov. Tucumán, Capital, 18–II–1955, *Singer T 2185* (LIL), *T 86* (MICH); San Javier, 12–I–1957, *Singer T 2870* (LIL).

This species belongs in the *Alcalina* group proper (Stirps *Alcalina:*

[2] We have one collection with a cortinoid veil, but this did not have the alkaline odor so that its identity is uncertain (*Singer T 1662*, MICH).

M. alcalina, chlorinella, niveipes, etc.), and according to the characters of the stipe appears to be closest to *M. niveipes* Murr. A comparison with this latter species—specimen collected by *Singer and Digilio* in June 1948 at Cheboygan, Mich., and determined by Dr. A. H. Smith—shows that the two species are not identical. The South American species differs in particular in its characteristic papilla. The spores are slightly narrower than in *M. niveipes.* The habit of fresh as well as dried specimens of both species is markedly different.

The question whether the type of this species and the specimens described in the preceding description are identical is not absolutely and positively decided, yet two facts appear to favor an affirmative decision, viz. (1) the type specimen found at the Herbarium Rick in Porto Alegre looks like the specimens collected by us and there are no data at hand to prove that there exists any appreciable difference between them, and (2) in a suburb of Porto Alegre, most probably very near the original locality, a collection has been made by me, in company of Father B. Rambo, and this specimen is undoubtedly identical with all the other collections. The only item which might cast some doubt on our interpretation is the original description published by Rick. Rick mentions an olive color outside the center of the pileus and the stipe is described as being much darker and shorter than we have ever observed it. The colors may be attributed to a deterioration of the carpophore before it was annotated, and the length of the stipe may be more variable than anticipated. But, at the moment, these discrepancies exist.

Mycena februaria Sing. spec. nov.

Pileo fusco, sulcato, convexo, umbilicato, 9-15 mm lato. Lamellis albis, arcuatis, decurrentibus, distantibus. Stipite castaneobrunneo, apice albo, glabro, levi, 10–18 × 0.8–1.2 mm. Oodore nullo. Sporis 8–10.8 × 5.3–5.5 μ, admodum debiliter amyloideis vel inamyloideis; cystidiis paucis, fusoideis; cheilocystidiis hyphosis, diverticulatis basi incrassatis; hyphis voluminosis, amyloideis, in epicute haud diverticulatis nec gelatinosis.

Pileus fuscous, *Malay* to *Madrid* (M & P 15–C–11/10), strongly sulcate to the umbilicus, glabrous, strongly convex, strongly umbilicate, not viscid, 9–15 mm broad.

Lamellae white, eventually cinereous-white, very broad, arcuate, decurrent, distant, intermixed, not intervenose. Spore print pure white.

Stipe chestnut brown (very deep colored: *Java br.* M & P 8–J–8 at the base, *casserole* M & P 7–H–11 above), and white at the apex, glabrous, slightly dull-shining, smooth, slightly tapering downwards, 10–18 × 0.8–1.2 mm, not insititious, with some mycelial fibrils at base, which form radiant strigose white aerial mycelium which is applicate to the substratum.

Context concolorous with the surfaces except in pileus, where it is whitish; odor none.

Spores 8–10.8 × 5.3–5.5 μ, ellipsoid to oblong, smooth, thin-walled and many with one large oil-drop, some very weakly amyloid, some inamyloid.

Hymenium: Basidia 24–27 × 6–8.7 μ, clavate, few seen with sterigmata, and then 1- or 2-spored. Cystidia few and not striking, 30–31 × 6.7–7.3 μ, fusoid, obtuse or acute, sometimes forked. Cheilocystidia and diverticulate hyphae making the edge of the lamellae strikingly heteromorphous, cheilocystidia like erect diverticulate and forked hyphae, only a few of them with slightly thickened basal cylinder or ventricosity, all hyaline.

Hyphae: some with, some (especially the broad somewhat thick-walled hyphae of the stipe) without clamp connections, all amyloid, voluminous in hymenophoral trama, pileus trama and especially the hypodermium, all hyaline except those of the hypodermium, which are colored by a brown intracellular pigment.

Covering layer of the pileus: Epicutis consisting of repent hyphae forming a cutis, not diverticulate, but external ones often with a colorless incrustation (artifact?), not gelatinized.

Occurring singly but gregariously on dead branches of a dicotyledonous tree in subtropical forest, fruiting in February.

Argentina: Prov. Tucumán, Parque de Aconquija, Sierra de San Javier, 17–II–1955, *Singer T 2184*, TYPE (LIL).

This omphalioid species belongs in the same group with such species as *Mycena cinerella*.

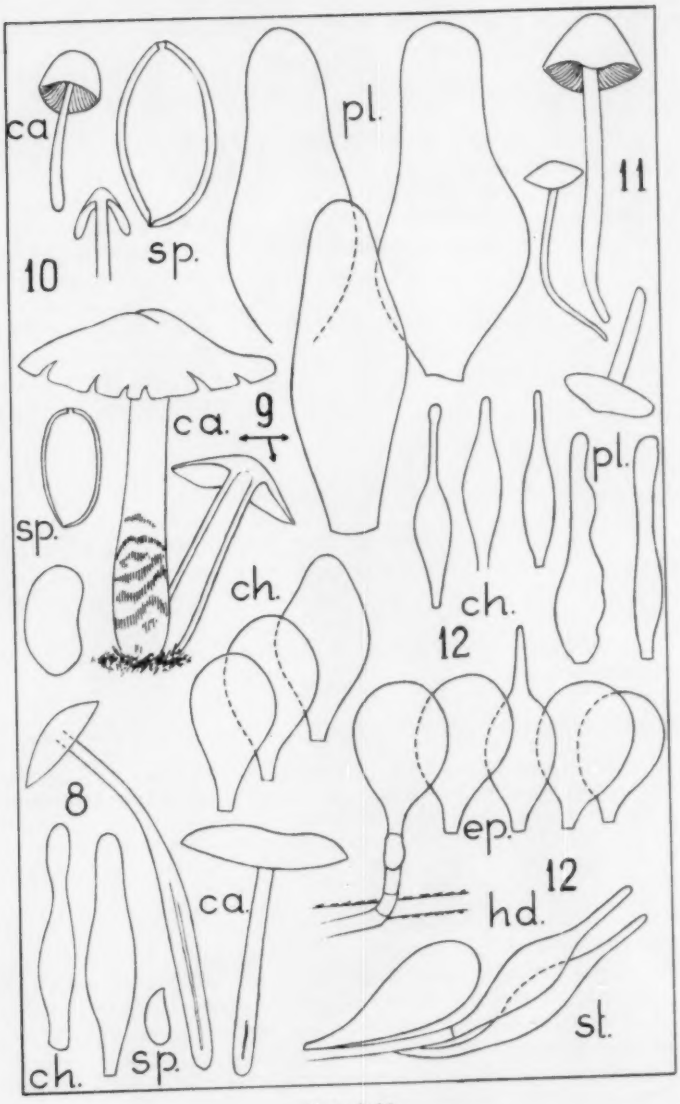
Mycena heroica Sing. spec. nov. FIG. 7

Pileo laete intenseque roseo, aetate pallescente, 3–14 mm lato. Lamellis albis, saepe intervenosis, adnexis vel (frequentius) decurrentibus. Stipite albo, glabro, haud insititio, 3–32 × 0.5–1.5 mm. Sporis 4.3–5 × 3–3.3 μ; cystidiis 25–34 × 6.7–9.3 μ, versiformibus, ad latera aciesque; dermatocystidiis stipitis magis vesiculosis quam cystidia; superficie stipitis hyphis diverticulatis ornata; hyphis sporisque inamyloideis; fibulis praesentibus.

Pileus of a bright and saturated pink (*Burmese gold, carnelian r., flamingo*, M & P 1–I–11, 2–I–11, 2–E–11, 3–C–11), pallescent from center outward in age, transparently striate in older smaller pilei, or else partly sulcate in age, or becoming sulcate all round, and often transparent at the same time, but not strictly hygrophanous, glabrous, convex, obtuse, eventually applanate, 3–14 mm broad.

Lamellae white, close to rather distant, narrow (about $\frac{1}{10}$ of diameter of pileus in breadth), horizontal to arcuate, adnexed to adnate or more often decurrent, intervenose or more rarely not anastomosing.

Stipe white or whitish, dry, glabrous, naked, smooth, sometimes



FIGS. 8–12.

curved (especially the short-stemmed forms), equal, subequal, central or slightly eccentric, 3–32 × 0.5–1.5 mm; mycelial tomentum white.

Context white; odor none.

Spores 4.3–5 × 3–3.3 μ, broadly ellipsoid, smooth or with occasional resinous hyaline patches, inamyloid.

Hymenium: Basidia 18.7–19.5 × 4.7–5 μ. Cystidia on sides of lamellae and on edges, versiform, varying from subvesiculose to ventricose or subulate, not strictly acute, but sometimes attenuated to a thin apex, very frequently septate in the middle, or in the upper third, and broader below septum, inamyloid, smooth and entire, rather numerous, 25–34 × 6.7–9.3 μ.

Hyphae: all inamyloid and with clamp connections, some hyphae of tramal layers very voluminous; oleiferous hyphae very conspicuous, especially in Melzer solution.

Covering layers: Epicutis of pileus a cutis consisting of repent filamentous hyphae, the outermost layer intermittently and obtusely diverticulate; intermittent diverticulations and, besides, some dermatocystidia occurring on stipe, most of them more vesiculose than the average hymenial cystidium.

On the ground, growing on dead vegetable matter, especially small sticks and needles of *Podocarpus parlatorei* and *Alnus jorullensis* var. *spachii* and on various species of *Eugenia* and allied Myrtaceae. Fruiting in summer and early fall.

Argentina: Prov. Tucumán, Las Lenguas, 20–II–1951, *Singer T 1314* (LIL). Taficillo, 6–I–50, *Singer T 811* (LIL), 22–II–55, *Singer T 2235*, TYPE (LIL).

This species is very close to *Mycena roseocandida* (Peck) Sacc.

Mycena sosarum Sing. spec. nov. FIG. 8

Pileo livido-lilaceo vel roseo-lilaceo, 20–30 mm lato. Lamellis albis, fortiter anastomosantibus, decurrentibus. Stipite lilaceo-livido, haud flavo. Carne raphaniolente. Sporis 7.3–10 × 4–4.7 μ, inamyloideis; cystidiis nullis, cheilocystidiis 26–43 × 6–8 μ subventricosis; hyphis epicutis inconstanter subdiverticulatis.

Pileus livid lilac or pinkish lilac (53–F–3 or 51–G–1 M & P), glabrous, slightly and shallowly but coarsely sulcate, convex, then depressed in the center, 20–30 mm broad.

FIGS. 8–12. FIG. 8. *Mycena sosarum* Sing. ca. carpophores; sp. spore; ch. cheilocystidia. FIG. 9. *Psathyrella macquariensis* Sing. sp. spores; ca. carpophores; pl. pleurocystidia; ch. cheilocystidia. FIG. 10. *Conocybe ambigua* (Kühner) Sing. ca. carpophores; sp. spore, both from Asiatic material. FIG. 11. *Conocybe halophila* Sing. carpophores. FIG. 12. *Agrocybe xerophytica* Sing. ch. cheilocystidia; pl. pleurocystidia; ep. epicuticular cells of the pileus; hd. hypodermium; st. elements of the surface layer of the stipe. (All spores × 1780; other microscopical drawings × 890; macroscopical drawings × 1.)

Lamellae white, strongly anastomosing, the anastomoses dense and varying from venose to lamellar, i.e., reaching the breadth of the through-lamellae, distant, decurrent.

Stipe lilaceous-livid (53–F–1, M & P) or darker, without any yellow at the base, hollow, equal or subequal, often compressed, splitting, or canaliculate, 36–63 × 2–3.5 mm, not insititious, conspicuous rhizomorphs absent.

Context white to concolorous with the surface; odor distinctly raphanaceous.

Spores 7.3–10 × 4–4.7 μ, at first obovoid, then oblong to cylindric, smooth, hyaline, inamyloid.

Hymenium: Basidia 22–30 × 7–8 μ, 1–4-spored. Cystidia none. Cheilocystidia 26–43 × 6–8 μ, somewhat ventricose below or in the middle, often with wavy outline or with several constrictions, hyaline, not very numerous.

Hyphae: amyloid in pileus, lamellae, and stipe; hymenophoral trama regular; trama of pileus and hypodermium elongate but often very broad, hyaline, forming a seemingly subcellular layer.

Covering layer: Epicutis of pileus a very thin layer of very thin, loosely arranged hyphae which are smooth or more rarely irregularly, inconstantly, and obtusely diverticulate, hyaline, later tending to collapse or being washed away, exposing the hypodermium in the older carpophores.

On the ground in subtropical forest, fruiting in summer.

This species differs from *M. violacella* (Speg.) Sing. in having white lamellae and lacking a yellow base, also in the presence of a raphanaceous odor. It differs from *M. anastomosans* (Sing.) Sing. (Florida)—probably not specifically different from *M. violacella*—in much stronger anastomoses of the lamellae and the color of the hymenophore.

Argentina: Prov. Tucumán, Rio de los Sosas, 4–II–1955, *Singer T 2128*, TYPE (LIL).

Amanita aestivalis Sing., Lilloa **22**: 387. 1951, ex Sing. spec nov.[3]

Pileo albo, haud flammato; notis ceteris *Amanitae brunnescenti* analoga species.

Pileus white without any brown or fuscous discoloration, sometimes slightly pale and sordid alutaceous on the disc, otherwise not showing

[3] The first publication of the binomial *Amanita aestivalis* (l.c.) provides the essential characters in the key (p. 391), but contains no Latin diagnosis. The reference to certain descriptions of *Amanita verna* published by North American mycologists and referring in reality to *A. aestivalis* cannot constitute the basis for a *nomen novum*.

any discolorations except for occasional brown-red shades from the discoloring flesh in old specimens, and without any innate striation or radiate color marks, moist to subviscid, drying to opaque or shiny, usually without any remnant of the thin evanescent volva, only rarely with irregular white patches forming a fragmentary thin fugacious covering over parts of the pileus, usually smooth, rarely with a short marginal striation (up to 4 mm), parabolic-obtuse or eggshaped, soon convex, umbonate or obtuse, eventually flattened or with uplifted margin, 54–83 mm broad.

Lamellae white, close, subventricose, moderately broad, with slightly fimbriate edges, free. Spore print pure white.

Stipe white, not or very minutely flocculose, subequal or slightly tapering upwards, stuffed, 90–160 × 9–16 mm; bulb broad and well developed, reaching a diameter of 33 mm or more, marginate with a rim; annulus white, membranous, fairly persistent, smooth or subsmooth, attached 12–17 mm from the extreme apex; volva strongly reduced and adnate to the outside of the bulb, projecting beyond the rim of the bulb in some cases (1–10 mm) as a thin, collapsing, fragile membrane, or from the beginning obliterated and scarcely noticeable, as in *A. brunnescens*, never cup-shaped or permanently free.

Context white, distinctly turning brown-red, pinkish brown or chocolate, or first brown, and then deep brown red ("Pekinese," "tortoise," "Java" M & P), but slowly so especially in age and in long-exposed wounds, somewhat less readily so than in *A. brunnescens*, and sometimes staining even the surface of the old stipes, not changing immediately on bruising, mild, inodorous or with a slight raphanaceous odor which becomes unpleasant and alliaceous in age.

Spores spherical, hyaline, smooth, thin-walled or almost thin-walled, amyloid, 7.8–8.8 μ in diameter, rarely larger and then sometimes subglobose but never more than 1 μ longer than broad.

Hymenium: Basidia as in *A. brunnescens*, e.g., 58 × 12.5 μ, 4-spored.

Chemical characters: H_2SO_4 on context reddish, but discoloration evanescent.—NH_3 and NH_4OH on annulus negative.—KOH on annulus negative or pale citron for a short while.—Phenol negative (one hour exposure).

On the ground in frondose and mixed woods, especially in oak woods with *Tsuga* or *Pinus*, or in beech woods with *Picea*, *Abies* and *Betula*, fruiting from late June until fall. Frequent from New England to Florida and west to Michigan.

U. S. A.: Innumerable collections from Massachusetts, also from New York, Virginia, preserved at FH.—*Mich.:* Cheboygan Co., various collections in summer 1953 (F), including one from east shore, Burt Lake, July 29, *R. Singer 899*, TYPE (F).

This species is generally misdetermined as *A. verna* in the Eastern States, but is occasionally considered a white form of *A. brunnescens*, which is closest. It differs, however, from the latter species in the constant absence of the fuscous radial stripes or innate fibrillosity, and numerous careful observations in places where the species is common, last in 1953, confirmed the author's belief that the species is divided from *A. brunnescens* without any transitions. Among the other white species, *A. verna* differs in ellipsoid spores, and *A. virosa* in more floccose stipe and well developed free volva as in *A. phalloides*, which differs aside from that also in green color. *A. verna, virosa,* and *bisporigera* are also common in Cheboygan Co., Mich., and were compared throughout the season.

Psathyrella kellermanii (Peck) Sing. comb. nov.

Galera kellermanii Peck, Journ. Mycol. **12**: 140. 1908.

Galerula kellermanii (Peck) Murr., N. Amer. Fl. **10**: 165. 1917.

For the macroscopical description and an illustration, I refer to the original description by Peck, l.c.

Spores 8.5–9.3(–11.5) × 4.7–6(–6.5) μ, ellipsoid, rarely somewhat reniform, smooth, brown when seen on lamellae with binocular, brownish hyaline under oil immersion, without metachromatic (pink) endosporium in cresyl-blue mounts, pseudoamyloid except for the pore region (but weakly so), with a non-truncate very narrow apical pore which is particularly distinct in the Melzer solution, without suprahilar depression, with homogeneous epi- and endosporium.

Hymenium: Basidia not studied. Cystidia on sides and edges of lamellae equally numerous, 28–40 × 11.5–14.3 μ, vesiculose-elongate ventricose, sometimes with a constriction underneath the apex, always broadly rounded.

Hyphae: all with clamp connections; hymenophoral trama apparently regular; all hyphae inamyloid.

Covering layer of pileus: Epicutis of the *Psathyrella* type, cellular, with somewhat compressed subisodiametric elements. Stipe with a surface layer consisting of spherocysts (an epithelium).

On the ground in a greenhouse, Columbus, Ohio (CU, part of TYPE collection).

This is a good species of *Psathyrella*, not a *"Galera"* in any sense. It is very rare in the United States, and obviously introduced with plant material.

Psathyrella macquariensis Sing. spec. nov. FIG. 9

Pileo hebeter brunneo, centro dilutiore, conico-applanato, 15–35 mm. Lamellis concoloribus, confertis, adnatis. Stipite flavo-cremeo, atrobrunneo-fibrilloso in parte

inferiore, subcartilagineo, fistuloso, neque annulato nec volvato, 25–40 × 0.7–0.8 mm. Sporis 9.5–10.3 × 6.2–6.8 μ, levibus, ellipsoideo-subreniformibus, griseis; basidiis tetrasporis; cystidiis numerosis, ventricosis, apice rotundatis et 10–15 μ diam., parte ventricosa 10.8–24.8 μ diam.; hyphis tramatis hyalinis.

Pileus dull brown, lighter in the central portion, conic-convex but soon flattened, becoming irregular from splitting marginal region, smooth and glabrous or almost so, 15–35 mm broad.

Lamellae dull brown, close, adnate.

Stipe yellowish cream and in lower half with appressed fibrillose dark brown mottling, fistulose, broader (ventricose) in lower portion than in the equal upper half which is glabrous or subglabrous, 25–40 × 7–8 mm; annulus and volva absent; veil not conspicuous.

Context fleshy, cartilaginous in the thin wall of the stipe.

Spores 9.5–10.3 × 6.2–6.8 μ, ellipsoid in face view, distinctly bean-shaped in profile, in KOH dark umber or gray, in Melzer's reagent reddish brown (pseudoamyloid), pallescent in concentrated H_2SO_4, with double wall and very distinct germ pore, smooth.

Hymenium: Basidia 22 × 8.2–9 μ, clavate, 4-spored. Cystidia 44–54 × 10.8–24.8 μ, numerous, on both sides and edges of lamellae, ventricose and broad below, slightly narrower in apical portion (10–15 μ diam.), broadly rounded above, short pedicellate in most, not or very little incrusted, inamyloid inside and in wall, hyaline, smooth, walls not thickened. Cheilocystidia like pleurocystidia, or more vesiculose, almost balloon-shoped, shorter than the cystidia, mostly about 27 to 30 μ long and as broad as pleurocystidia.

Hyphae all hyaline (incl. hymenophoral trama which is regular).

Covering layer of pileus; epicutis distinctly cellular.

On humus, cespitose, fasciculate.

Australian Subantarctic: Macquarie Island, Tent Hill, 15–XI–1951, *Bunt MB/51/B6*, TYPE (LIL).

Conocybe ambigua (Kühner) Sing., Lilloa **22**: 485. 1949 (1951) ex Sing. (hic). FIG. 10

Conocybe siliginea var. *ambigua* Kühner, Galera, p. 106. 1935, n. nud.

Galera ambigua (Kühner) Lange, Dansk Bot. Ark. **9** (6): 38. 1938, n. nud.

Pileus brown or ochraceous brown like wheat-bread crust, or ochraceous, with paler margin, hygrophanous, dirty pale ochraceous faded (usually about *raw sienna* M & P 13–L–10, with paler places especially between striae when fresh, much duller and paler when dry), transparently striate, usually over ⅔ of the radius or more, glabrous, or very finely pubescent (under a lens) when fresh and young, smooth, or some-

times sulculate along the striae, obtusely conic or campanulate, more rarely campanulate-convex or hemispherical, 6–20 mm broad.

Lamellae brownish ocher, almost pallid when young and immature, not ventricose or slightly so, close, 2–3 mm broad, ascending, adnexed to adnate.

Stipe pallid or whitish, often becoming pale melleous with the lower portion almost concolorous with pileus in age, but in some individuals almost entirely pallid with brownish ground color in age, pallid pruinose, at least at apex, and more villous-pruinate below, often minutely longitudinally striatulate, 25–50 (acc. to Kühner also up to 70) × 1 mm.

Context very thin, fragile. Odor none or not significant.

Spores 10–18 × 6–9.5 μ, most frequently 11.5–15 × 6.5–9.5 μ, narrowly to broadly ellipsoid, smooth, very variable in their quotient (length/breadth), with distinct truncate germ pore.

Hymenium: Basidia 20–30 × 10–11.5 μ, 2-spored, fewer 3-spored, exceptionally 1-spored. Cheilocystidia 16–24 × (6–)7.8–8.2(–10.4) μ, sharply capitate, neck 1.7 μ diam., capitulum 2.6–5 μ, mostly about 3.7 μ diam. Ammonia reaction negative (no needle crystals formed).

Hyphae with clamp connections.

Covering layer of pileus a palisade of short cells; of stipe formed by intermittent bunches of dermatocystidia, with and without capitate apex (those with capitate apex analogous to the cheilocystidia, with a capitulum of about 5 μ diam., those without having ellipsoid-ventricose to subcylindric or ampullaceous shape and measuring e. g. 15 × 10 μ), and, aside from these, numerous long awl-shaped hairs; capitate dermatocystidia rather scattered (minority among dermatocystidia) in the middle of the stipe and becoming very exceptional below, but more numerous on apex.

On the earth among herbs and grasses, in open meadows, pastured mountain slopes, in parks under scattered trees, and in greenhouses, fruiting from late spring until early fall, in greenhouses practically any time of the year, mostly singly.

Afghanistan: West slope of the Scher Darwasah near Kabul, 1800 m alt., with *Draba verna* and *Veronica* sp., 13–IV–1951 (LIL). Also numerous collections in Europe (see Acta Inst. Bot. Ac. Sc. U.R.S.S. II. **6**: 442. 1950, sub *Conocybe siliginea* var. *ambigua*, Kühner, l.c. and Lange, l.c.).

This and the following species are the first representatives of this genus in Afghanistan.

We consider this form an autonomous species, different from *Conocybe siliginea*, as a variety of which it was first published, and from

C. ochracea and *plumbeitincta*. Kühner & Romagnesi and Lange agree now with this new concept.

There is some confusion about the correct specific names to be attributed to each of the species under consideration.

C. sienophylla (Berk. & Br.) Sing., Sydowia **9**: 402. 1955 = *Agaricus siennophyllus* Berk. & Br., Jour. Linn. Soc. Bot. **11**: 545. 1871 = *Conocybe siliginea* var. *ocracea* Kühner, Galera p. 101. 1935. The *G. siliginea* in the sense of J. Schäffer (non Fries) is a grayish form of *G. siennophylla* forma *cinerascens* Sing.

C. plumbeitincta (Atk.) Sing., Sydowia **4**: 137. 1950 = *Galerula plumbeitincta* Atk.; see below.

C. siliginea (Fr.) Sing., Act. Inst. Bot. Ac. Sc. U.R.S.S. II. **6**: 443. 1950 (sensu Bres.) = *Galera siliginea* (Fr.) Quél. = *Conocybe Rickeni* Kühner nom. nud.[4] is the common species, mostly found in dehydrated state and 2-spored. The Friesian *A. siligineus* can be interpreted with more probability as the species so called by Bresadola than in the sense of J. Schäffer adopted by Kühner (see Lange, Fl. Agar. Dan. **4**: 36 (above). 1939, and Singer, Sydowia **4**: 137. 1950).

[4] According to the new version of the International Rules, "on and from 1 Jan. 1935, names of new taxa of recent plants, the bacteria excepted, are considered as validly published only when they are accompanied by a Latin diagnosis." Consequently, the new taxa published in Kühner's fundamental *Galera* monograph (some time during 1935) are not validly published. Therefore, I consider it essential to provide Latin diagnoses for the following conocybes:

C. mesospora Kühner. Pileo aurantiofulvido 17–45 mm lato; stipite haud radicato, dermatocystidiis capitatis obsito, cheilocystidiis parvis, parvi-capitatis; sporis 7.5–10 × 4.2–5.7 μ, poro manifesto. Circum Lutetiam (P).—Recenter redefinita ut *C. brunneola* nom. nud.

C. spiculoides Kühner. A *C. rickeniana* Sing. = *spicula* sensu Ricken differt sporis abnormaliter pallidis, membrana subtenui, poro subnullo. Lutetiae (P).

C. semiglobata Kühner. A *C. tenera* differt pileo semiglobato, mox dehydratato. Circum Lutetiam (P).

C. macrocephala Kühner. A *C. tenera* differt sporis 8.5–10 × 5.5–6 μ capitulis cheilocystidiorum saepe usque ad 5.7 μ diam. Lutetiae (P).

C. ochracea (Kühner ex) Sing. Sporis 9.5–10.5 × 5.5 μ, interdum minoribus, nonnullis interdum maioribus; stipite dermatocystidiis capitatis destituto; pileo typice ochraceo. Lutetiae (P).

C. AMBIGUA (Kühner ex) Sing. Pileo brunneo vel ochraceo, longe striato, 6–20 mm lato; stipite pro ratione pallido, dermatocystidiis capitatis, decapitatis, nec non pilis in centro suo ornato; sporis 10–18 × 6–9.5 μ. In et prope Lutetiam (cf. descriptionem originalem hoc loco additam).

C. cylindracea R. Maire & Kühner. Pileo elongate campanulato vel ellipsoideo vel cylindraceo; stipite aetate brunnescente, dermatocystidiis capitatis destituto. La Régaïa, Algeriae.

CONOCYBE PLUMBEITINCTA (Atk.) Sing., Sydowia **4**: 131. 1950.
Galerula plumbeitincta Atk., Proc. Amer. Phil. Soc. **57**: 372. 1918.

Pileus with a tinge of gray, especially when young, lubricous when young and moist, even or substriate when dry, glabrous, at first convex, then campanulate, always obtuse, 10–15 mm broad.

Lamellae ochraceous cinnamon, ventricose, rather broad, subdistant, adnate.

Stipe white, becoming dingy like the pileus, shining, mealy at apex, substriate below, straight or somewhat curved, hollow, equal.

Context white in pileus and stipe, subrigid, cartilaginous and rather tough in the stipe; odor not mentioned.

Spores 12–13.5 × 7.8–8.2 μ, mostly about 13 × 8 μ, very deeply rusty fulvous (well colored, NH_4OH), with distinct truncate germ pore, ellipsoid.

Hymenium: Basidia 4-spored. Cystidia none; cheilocystidia on heteromorphous edge, 19.3–21.5 × 5–7.5 μ, varying from ampullaceous to capitate, many subcapitate, few truly capitate as in most species of *Conocybe* and few truly ampullaceous and non-capitate without any thickening in the apical region, hyaline.

Covering layer of the pileus consisting of a hymeniform layer of obpiriform cells, some of them with brownish lower portion, others entirely hyaline, 11.5–30.5 × 8.7–21.5 μ; dermatocystidia here none. On stipe numerous cystidioid bodies about the middle and also on apex of stipe, all vesiculose, some ampullaceous, none capitate; there are also numerous hyaline long hyphous hairs.

On dunghills in spring.

U. S. A.: Michigan, Cascade Glen, Ann Arbor, June 9, 1906, *C. H. Kauffman 565*, TYPE (CU).

The macroscopic description is copied from notes accompanying the type specimens and strictly identical with those indicated by Kauffman, who must have found this fungus in faded condition. This redescription proves that there are some minor differences between this and *C. ochracea* fa. *cinerascens* Sing., and they should be considered different species. Although non-capitate cheilocystidia can be found in *C. ochracea*, the large percentage of these in the type of *C. plumbeitincta* seems to show that the two species are microscopically different. Besides, the tough character of the stipe emphasized by Kauffman who, even in his youth, was a keen observer, should not be neglected. Furthermore, it seems that the true color of the pileus and stipe is nearly white rather than gray, and consequently the species would become comparable with *C. siliginea* (sensu Bres.) rather than with *C. ochracea*, and should be compared particularly with the 4-spored form described by Kühner. But

this latter is not said to have toughish stipe, and its spores are smaller than those of *C. plumbeitincta*. The 4-spored form of *C. siliginea* described by Singer & Digilio has not only smaller spores but also different colors. And even if the Michigan species were conspecific with the 4-spored forms described by these authors, their conspecificity with *C. siliginea* fa. *bispora* (typica) remains extremely doubtful and has been assumed only tentatively by those who have collected them. Since a transference of *C. plumbeitincta* had already been made on the mistaken assumption that it is identical with *C. ochracea* Kühner (over which it would have considerable priority), it seems permissible to maintain the Michigan species as autonomous.

Conocybe halophila Sing. spec. nov. FIG. 11

Pileo quam in *C. ochracea* crassiore, griseolo-fusco, centro obscuriore. Stipite albello vel brunneolo, fortiter longitudinaliter striato, basin versus attenuato, crassiusculo ad apicem. Sporis 8.5–11.5 × 5.7–7.2 μ; dermatocystidiis capitatis nullis in stipite.

Pileus grayish brown, in the center deeper colored, finely striate over most of the radius, not reticulate-rugose, campanulate, about 17 mm broad.

Lamellae rusty brown, wavy, subclose to close, adnexed.

Stipe whitish to brownish, strongly longitudinally striate, especially on the apex, fibrillose-pruinate all over, tapering downward to a non-bulbous base, 20–50 × 4 mm.

Context comparatively thick in pileus and stipe, not fragile.

Spores 8.5–11.5 × 5.7–7.2 μ, ellipsoid, smooth, with distinctly truncate broad germ pore, well pigmented in NH_4OH and detergent, very deep rust-color in KOH.

Hymenium: Basidia 21.3–28 × 9.2–10 μ, 3–4-spored. Cystidia none; cheilocystidia as in *C. ochracea*. No needle crystals in ammonia mounts. Some pseudoparaphyses present at edge of lamellae.

Hyphae with clamp connections. Hyphae of stipe parallel, thin-walled.

Covering layers: Epicutis of pileus cellular-hymeniform. Dermatocystidia in the middle of the stipe all vesiculose to vesiculose-mucronate, but none with capitulum, hyaline, e. g. 10 × 5.7 μ, forming bunches (responsible for the pruina), and also some hair-like elements present, these thickened at the base and acute above, strongly awl-shaped.

On the ground in a salt meadow, spring.

Afghanistan: West of Butschak along the road from Kabul to Nimla, 1740 m alt., 22–IV–1951. *Gilli CXXXIII/10*, TYPE (LIL).

This saline species of central Asia differs from the closely allied

C. ochracea mainly in habit and the more strongly striate stipe, and it differs besides from all forms or species which were subordinated to "*C. siliginea*" (= *C. ochracea*) by Kühner in 1935 either in color or size.

Bolbitius tucumanensis Sing. spec. nov.

Pileo fusco, centro albo, glutinoso, pectinato, glabro, 36–44 mm lat. Lamellis brunneis, angustis, adnexis vel liberis, confertis. Stipite albo, floccoso, basin versus incrassato, 75–95 × 4–5 mm. Sporis in cumulo obscure sordideque ferrugineis, s. m. 18–20.7 × 7–8.7 μ, ellipsoideis, ferrugineis, levibus, poro germinativo bene evoluto praeditis; cheilocystidiis ventricosis; hyphis defibulatis.

Pileus fuscous (*broncho* then pallescent to *auburn*, M & P 8–E–12 and 7–C–11), with whitish disc, glutinous, especially when young, pectinate densely and over most of the pileus in the manner of the *Coprini*, glabrous, campanulate, with straight margin, the latter at first applicate to the stipe, later pileus becoming expanded and eventually applanate, 36–44 mm broad.

Lamellae brown (*cochin* to *burnt umber*, M & P 7–A–12 to 15–A–12), crowded or close, narrow, adnexed or free. Spore print not bright ochraceous-rusty or fulvous-rusty but a deep dusky ferrugineous (*cocoa*, M & P 7–E–12, in thick layer *Mandalay*, M & P 8–L–12 to *burnt umber*, M & P 15–A–12).

Stipe white, floccose, tapering upwards, 75–95 × 4–5 mm.

Context white, unchanging, inodorous.

Spores 18–20.7 × 7–8.7 μ, ellipsoid, rusty, smooth, thick-walled, with complex wall, with distinct truncate germ pore, much like *Conocybe* spores.

Hymenium: Basidia 18–21.5 × 14 μ, 2- and 4-spored. Cystidia none. Cheilocystidia e. g. 25 × 12.7 μ, ventricose. Pseudoparaphyses 13.7–18.8 × 12–14.5 μ, vesiculose-clavate, hyaline, numerous.

Hyphae without clamp connections.

Gregarious on horse dung in a ravine, fruiting in summer.

Argentina: Prov. Tucumán, Quebrada La Higuera, 25–I–1955, *Singer T 2081*, TYPE (LIL).

This may be very close to *Galera versicolor* Peck from South Dakota, but the color of the pileus is different in the North American species, and the surface is said to be merely "moist or slightly viscid."

AGROCYBE FIRMA (Peck) Sing. var. **tucumana** Sing. var. nov.

Pileo dermatocystidiis destitutis. Ceterum *A. firmae* var. *firmae* simillima varietas.

Pileus in the center *coffee* (M & P 15–A–11), margin pale brownish cream (*Leghorn*, M & P 10–D–3), not more hygrophanous than *A. aegerita*, margin not striate or sulcate, glabrous all over, weakly rugose-

sulcate on one side of the pileus, convex with depressed center, 22–23 mm broad.

Lamellae *bronze* (M & P 14–L–9), broad, distant, separating, adnate. Spore print very much like that of *A. aegerita*, i.e., *clove* (M & P 15–C–12).

Stipe much paler than the pileus in dried condition because of a pallid fibrillosity or pruinosity in the upper third which makes the apex sordid white, below similar in color to the pileus but paler, subequal or with thicker base, 38 × 3–5 mm; veil indistinct, annulus none.

Spores 6.5–7.3 × 4.5–4.7 μ (from 4-spored basidia; 9.3 × 6 μ or more elongate from the 1–3-spored basidia), well colored, with complex smooth wall, almost rusty ochraceous, with or without a germ pore, the ones with germ pore having a very narrow and often indistinct apical discontinuity, never a truncate pore.

Hymenium: Basidia 26.5–34.5 × 5–7.3 μ, most 4-spored, fewer 2-spored, very few 1- or 3-spored. Cystidia on sides of lamellae 30–44 × 13–15.3 μ, ampullaceous with broad (e. g. 10 μ diam.) upper portion, rarely broadly cylindrical or vesiculose, the mucro rarely subcapitate, hyaline. Cheilocystidia none.

Hyphae with clamps. Hymenophoral trama regular.

Covering layers: Epicutis of pileus consisting of a hymeniform layer whose elements are obpiriform, or vesiculose-clavate, rarely globose, pedicellate, yellowish hyaline, base in certain places on the pileus with a rusty epicellular incrusting pigment which is somewhat more abundant in the hypodermium; the latter filamentous but irregular and consisting of inequal but elongated hyphae. Surface of stipe with bunches of dermatocystidia which are hyaline and mostly ventricose to subventricose-clavate, very rarely vesiculose or subcapitate, 33–51 × 10.7–16 μ. On the pileus, dermatocystidia were not found.

On humus under dead root in subtropical forest, summer fruiting.

Argentina: Prov. Tucumán, Rio de los Sosas, 4–II–1955, *Singer T 2130*, TYPE (LIL).

The absence (or scarcity) of the dermatocystidia of the pileus and the shape of the dermatocystidia of the stipe and a few other minor characters of doubtful constancy seem to suggest that the collection under study belongs to a South American race, not fully identical with the type.

Agrocybe xerophytica Sing. spec. nov. FIG. 12

Pileo brunneo, haud hygrophano, haud viscido, glabro, convexo, dein applanato, subumbonato, 8–20 mm lato. Lamellis argillaceo-brunneis, latis. Stipite albo vel pallide alutaceo, farinoso dein fibrilloso, solido, aequali, 12–20 × 1.2–3.8 mm; velo subnullo. Carne alba, inodora. Sporis 6.7–8.7 × 4.7–6 μ, poro germinativo minutissimo, non truncato. Cystidiis paucis, cheilocystidiis fusoideis vel ampullaceis (apice tenerrimo).

Pileus ochraceous brown (center *Yucatan*, M & P 12–L–9, margin *samovar*, M & P 12–K–7), not hygrophanous, not viscid, glabrous, naked, eventually slightly rivulose-rimulose in some specimens but usually with entire surface, not sulcate or striate, convex then flat, subumbonate, 8–20 mm broad.

Lamellae argillaceous brown, broad, subclose to subdistant, sinuate-adnexed, later adnate. Spore print rust brown on surface of pileus.

Stipe white to pale buffish, mealy, then fibrillose, solid, equal, 12–20 × 1.2–3.8 mm; veil only a white pubescence at extreme margin of very young specimens, soon disappearing.

Context white or whitish, fragile, inodorous.

Spores 6.7–8.7 × 4.7–6 μ, ellipsoid, smooth, well pigmented (melleous ochraceous-brown), with a tiny non-truncate germ pore, or some spores without any discontinuity at the apex.

Hymenium: Basidia 26.7–30 × 5.5–6.7 μ, hyaline, thin-walled, clavate, more often 4-spored than 2-spored. Sclerotized "basidia" (since they are apparently sterile, they should be considered cystidioles) like the others but brownish, thick-walled, with (2–)4 sterigmata, scattered on edge and sides. Cystidia 36–39 × 5.3–9.3 μ, cylindrical to ampullaceous, thin-walled, scattered and rather rare. Cheilocystidia 24.7–30 × 3.3–7.3 μ, fusoid to ampullaceous, the apical part very thin (1.7 μ diam.) and obtuse to acute, sometimes with some inconspicuous crystalline incrustation at apex, hyaline, thin-walled, not forming a heteromorphous edge since they are interrupted by numerous basidia.

Hyphae with clamp connections. Hymenophoral trama regular.

Covering layer: Epicutis of pileus consisting of vesiculose or vesiculose-clavate pedicellate elements which form a hymeniform layer, rising from some erect hyphal chains of lesser diameter which, in their turn, rise from a cutis of repent hyphae (hypodermium), these hyphae strongly incrusted by a brown pigment. Elements of the epicutis e. g. 30 × 14 μ, with brownish membrana pigment; some narrower elements between them (dermatocystidia) similar to the cheilocystidia and with narrow apical excrescence; in abnormal cases (i.e., when several of these dermatocystidia are crowded together, apparently a consequence of wounding) filamentous appendages of the dermatocystidia may form peg-like formations which project beyond the general surface of the pileus.

On a bare earthy sandy bank of a dry river bed shaded by leguminous and other trees in subxerophytic mountain forest, growing singly but in large populations. Fruiting during the summer rains.

Argentina: Prov. Tucumán, hills above San Pedro de Colalao, 21–I–1955, *Singer T 2053*, TYPE (LIL).

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MORPHOLOGY AND TAXONOMY OF ALTERNARIA CUCUMERINA

CURTIS R. JACKSON AND GEORGE F. WEBER[1]

(WITH 2 FIGURES)

The Alternaria leafspot disease of cucurbits, caused by *Alternaria cucumerina* (E. & E.) Elliot, is an important disease in areas of the United States where muskmelon and watermelon are commercially grown. The disease has been known by several common names such as leaf blight, target leaf spot, Alternaria leaf blight, and Macrosporium blight. Characteristic symptoms appear only on the foliage (FIG. 1). The name Alternaria leafspot will be used hereafter.

Several species of *Alternaria* and *Macrosporium* have been cited as the causes of leafspot diseases of cucurbits. This is a report of a study to determine the species of *Alternaria* associated with cucurbit leafspot diseases in Florida. The morphology of the fungus, size range of conidia, and a comparison with reported morphological and pathological characterizations are presented.

REVIEW OF THE LITERATURE

Alternaria cucurbitae Let. and Roum. has been cited as the cause of a disease of cucurbits by Boughey (**1**) and Schwarze (**13**). Elliot (**2**), in his study of the genus, did not include *A. cucurbitae* because he was unable to obtain sufficient material. Peglion (**9**) believed *A. cucurbitae* to be distinct from the pathogen he described as the cause of a leafspot disease of cucurbits, principally on the basis of the great difference in spore-body diameter. Although *A. cucurbitae* may be regarded as a valid species, it is not the causal agent of the leafspot disease of cucurbits most frequently reported throughout the world.

In 1893, Peglion (**9**) described *Alternaria brassicae* f. *nigrescens* Pegl. as the cause of a disease of muskmelons in Italy. He gave conidial size as 60–80 × 14–18 μ and incorrectly described conidial shape as clavate. In a second report Peglion (**10**) revised the dimensions to 100–160 × 14–20 μ, reaching 200 μ in length when the filiform tip was

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greatly elongated. This revision apparently indicated that the original description was based only on spore-body length and that the revised length range resulted from the inclusion of beaks in the measurements. Later Peglion (**11**) gave a diagnosis which combined his revised conidial dimensions with the original description. Saccardo (**12**) listed *A. brassicae* f. *nigrescens* as a variety instead of a form as originally described and, apparently following his concept, this category has been used sub-

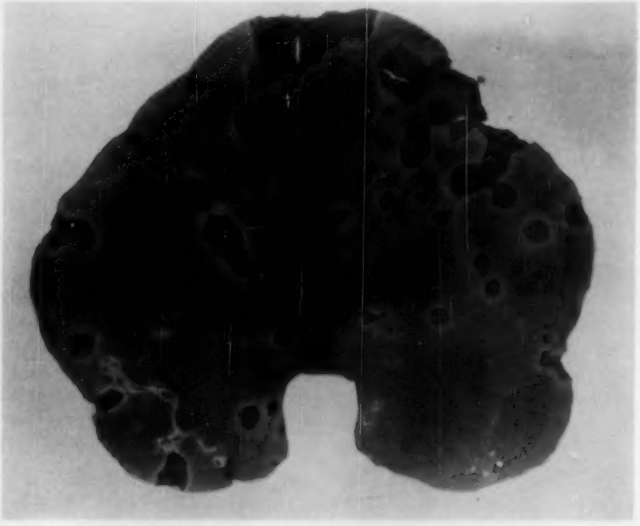

FIG. 1. Symptoms of Alternaria leafspot on Rio Gold cantaloupe leaf.

sequently. Neergaard (**7**) measured 10 conidia which were taken from the type material and found that the conidial body averaged 72.6 × 16.9 μ.

Ellis and Everhart (**3**) described *Macrosporium cucumerinum* E. & E. in 1895 from infected muskmelon leaves. They made a clear distinction between the conidial body and beak but mistook the latter for pedicels and gave the spore shape as clavate. Elliot (**2**) proposed an emendation to the circumscription of the genus *Alternaria* which allowed non-catenulate species to be included in the genus. He proposed a new combination *Alternaria cucumerina* (E. & E.) Elliot and considered *A. brassicae* f. *nigrescens* as a synonym. Nattrass (**6**) compared *A. cucumerina* with a specimen of *A. brassicae* var. *nigrescens* as distributed

in Briosi and Cavara's "Fungi Parassiti" and concurred with Elliot's proposal that the latter fungus was identical with *A. cucumerina.*

Neergaard (**7**) examined specimens of *A. brassicae* var. *nigrescens* and concluded that the fungus was distinct from *A. brassicae* (Berk.) Sacc. and should be considered a species. He proposed the combination *A. nigrescens* (Pegl.) Neerg. but did not examine *A. cucumerina,* since this species was not included in his study. TABLE 1 presents a compila-

TABLE 1

COMPARISON OF CONIDIA MEASUREMENTS IN MICRONS AS REPORTED BY VARIOUS AUTHORS

Name	Length	Width	Cross septa	Citation
A. brassicae f. *nigrescens*	60–80	14–18	6–8	Peglion (**9**)
A. brassicae f. *nigrescens*	100–160*	14–20	6–8	Peglion (**10**)
A. brassicae f. *nigrescens*	100*	14–20	6–12	Saccardo (**12**)
A. brassicae f. *nigrescens*	100–130*	20–26	—	Nicolas (**8**)
A. brassicae f. *nigrescens*	23–110**	8–40**	—	Elliot (**2**)
A. brassicae f. *nigrescens*	54–90	14–22	5–9	Neergaard (**7**)
M. cucumerinum	30–75	15–25	3–8	Ell. & Ev. (**3**)

* Measurements presumably included beaks.
** Data taken from graphs with modes about 38–75 × 17–20 μ.

tion of measurements made by various authors. Elliot's (**2**) measurements were made from an isolate identified as *A. brassicae* f. *nigrescens* which he believed to be synonymous with *A. cucumerina.*

MATERIALS AND METHODS

Conidia and conidiophores of *A. cucumerina* used in all measurements were taken from 9- to 12-day-old cultures incubated in darkness at 28° C and from lesions resulting from infection of host plants. All isolates were collected in Florida from infected muskmelon, squash, and watermelon foliage and were derived from single-conidium isolations. The fungus was cultured in petri dishes on 18% "V-8" vegetable-juice agar in all cases. Samples were taken from the surfaces of cultures or from natural media, i.e., sporulating lesions on the hosts. Conidia or conidiophores to be measured were mounted on microscope slides and selected at random. Only conidia which contained at least 1 longitudinal septation were included. Septations that were visible in the uppermost plane of focus were counted; those which were continuous in approximately straight lines, even though intersecting several other septations, were counted only once.

The greatest diameter of the conidial body and the diameter of the

beak at its midpoint were recorded. The conidial body and beak were treated separately in all measurements, since the length variability of beaks is considerably greater than that of the conidial body. The cri-

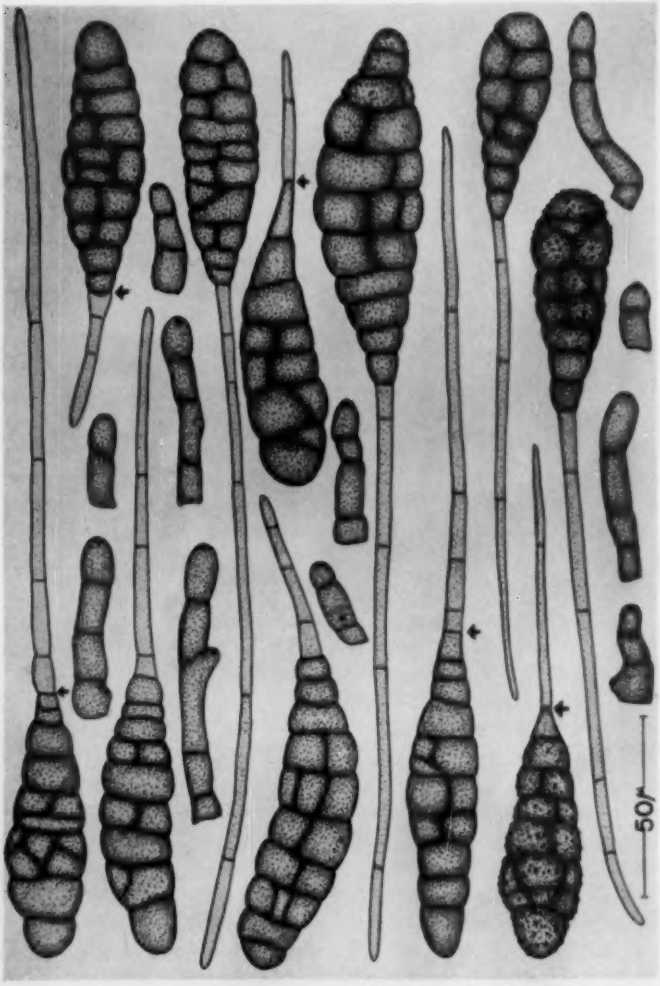

FIG. 2. Conidia and conidiophores of *Alternaria cucumerina*. Arrows show points at which distinction was made between conidial beaks and bodies.

TABLE 2

CONIDIAL BODY SIZE IN MICRONS TAKEN FROM SAMPLES OF SIX ISOLATES FROM MUSKMELON (M), SQUASH (S), AND WATERMELON (W)

Isolate	Medium	Mean size	Coefficient of variation*	
			Length	Width
M-20	V-8 agar	57.4 × 18.3	18.0	12.0
M-20	Muskmelon	86.5 × 18.8	19.6	12.6
M-35	V-8 agar	66.0 × 24.8	21.4	17.1
M-35	Muskmelon	73.0 × 20.0	18.6	12.7
M-36	V-8 agar	52.8 × 21.4	14.7	12.3
S-36	Squash	56.6 × 18.1	24.8	22.8
S-36	Watermelon	71.3 × 18.1	14.5	9.6
W-37	V-8 agar	59.7 × 21.1	17.3	13.1
W-37	Watermelon	76.1 × 21.0	20.0	15.6
M-38	V-8 agar	64.9 × 19.4	22.9	17.6
M-38	Muskmelon	79.9 × 18.5	10.0	14.0
M-41	V-8 agar	56.1 × 20.8	19.8	16.4
M-41	Muskmelon	66.4 × 18.1	15.1	13.7

* Coefficient of variation was calculated as standard deviation/mean × 100.

teria used to define the extent of the conidial body are similar to those employed by Neergaard (7). Color is the most valuable criterion, as the beaks are usually uniformly hyaline and the point of union with the darker body can be distinguished readily. The conidial body tapers rather abruptly to the junction with the beak and beyond this point the beak diameter becomes only slightly smaller throughout its length. A few terminal cells of the body of some conidia have approximately the same diameter as the base of the beak. These cells have been included in body length when their color was similar to that of the body. Occasionally, the cell wall of the apical body cell projected convexly into the lowest beak cell and this occurrence has been used in conjunction with other features mentioned to define the extent of the body. FIG. 2 shows examples of the conidiophores and of distinctions between beak and body in situations most frequently encountered.

RESULTS

Mean conidial-body sizes for 6 of the 11 isolates used in this study are presented in TABLE 2. Sample sizes were either 50 or 85 conidia for V-8 agar samples and 35 or 50 conidia for natural media samples. Standard deviations of the samples ranged from 7.7 to 17.0 μ for length and 1.7 to 4.9 μ for width. Mean conidial-body length and width were not excessively variable among isolates. Judging from the coefficients of variation, which permit direct comparisons between samples, body

width generally was less variable than body length. There was no consistent indication that body dimensions of conidia taken from V-8-agar cultures were more or less variable than those from natural media.

An indication of mean dimensions and length variability of beaks was obtained by taking samples of 35 conidia each from natural media and from V-8-agar sources of isolates M-20, S-36, and W-37. Mean dimensions of beaks were within the range of 106.0–217.4 × 2.2–2.7 μ for the 6 samples. Standard deviations for length were from 44.0 to 74.4 μ and the standard errors of the means for length were all less than 12.6 μ. The coefficients of variation for beak length were from 2–5 times larger than corresponding values for body length.

Data relating to the range of transverse and longitudinal septations were derived from the samples used to obtain beak dimensions. These data, conidiophore dimensions, and other pertinent morphological data are presented below.

DISCUSSION

The circumscriptions of *A. brassicae* f. *nigrescens* by Peglion (**9**, **10**) and *M. cucumerinum* by Ellis and Everhart (**3**) are similar in many details. The reported size ranges of conidia of these species as presented in TABLE 1 cannot be compared directly since Peglion (**10**), Saccardo (**12**), and Nicolas (**8**) apparently included beaks in conidial measurements. Detailed information was not given by any authors listed in TABLE 1, except Neergaard (**7**), regarding sample sizes and sources, and age of conidia included in the samples. The ranges of conidial body and beak dimensions as determined in the present study by direct measurements and as calculated from derived statistics are sufficiently large to include all ranges of conidial dimensions given by previous workers. The differences in the size ranges shown in TABLE 1 may be regarded as being due to variation in sampling methods used by the cited workers.

Alternaria brassicae f. *nigrescens* and *A. cucumerina* have been noted frequently as the cause of a cucurbit leafspot disease but distinctions between the symptoms have not been made. Peglion's (**9**) description of muskmelon symptoms is similar to those reported by many investigators, including the symptoms reported by Ellis and Everhart (**3**). Reported host ranges also agree with few exceptions. The writers have not examined the type specimens of either fungus. However, in view of the overlapping morphological features and the similarity of reported symptoms and host ranges the writers concur with Elliot (**2**) and

Nattrass (**6**) in the synonomy of *A. brassicae* f. *nigrescens* and *A. cucumerina*.

Although form *nigrescens* was described in 1893 and *M. cucumerinum* in 1895, no questions of the priority of the former name is involved, since a name or epithet has no priority outside its own rank according to Article 60 of the International Code of Botanical Nomenclature (**5**). When Elliot (**2**) formed the combination *A. cucumerina* he used the appropriate derivation of the species name of the former combination. The combination *A. nigrescens* which was proposed by Neergaard (**7**) is superfluous since it was based on *A. brassicae* f. *nigrescens* (**4**). The correct name of the Alternaria leafspot pathogen is *Alternaria cucumerina* (E. & E.) Elliot, Am. Jour. Bot. **4**: 439. 1917. The following synonyms are recognized: (1) *Macrosporium cucumerinum* E. & E., Proc. Acad. Nat. Sci. Phila. **1895**: 440. 1895; (2) *Alternaria brassicae* f. *nigrescens* Pegl., Riv. patol. veg. **1**: 297. 1893 (also cited by Saccardo (**12**) as *A. brassicae* var. *nigrescens* Pegl., Riv. patol. veg. **1**: 296. 1893); and (3) *Alternaria nigrescens* (Pegl.) Neerg., Danish Spp. Alternaria and Stemphylium, p. 232. 1945.

Elliot (**2**) did not redescribe *A. cucumerina*, and the only extant descriptions are those of Ellis and Everhart (**3**) and Peglion (**9, 10**). To clarify the circumscription of the taxon, the following description, which is based on Florida specimens, is proposed as an emendation of the present descriptions. All data were obtained from structures taken from host material unless otherwise specified. Conidial body dimensions were derived from 680 measurements of 7 isolates.

Hyphae in culture hyaline to olivaceous, branched, irregularly septate, 4–9 μ in diameter, white to dark olivaceous in mass; conidiophores maculicole, epiphyllous and hypophyllous, fasciculate to solitary, light to dark brown, geniculate or straight, 15–72 × 5–7 μ, 1–6-septate with slightly enlarged bases; conidia mostly apical on conidiophores, light brown, ovoid to obclavate with few longitudinal septations when young, becoming dark brown, obclavate to fusiform-obclavate with 1–10 longitudinal septations when mature; conidial body smooth to papillate, 33.6–129.4 × 13.4–33.6 μ, averaging 56.6–86.5 × 18.1–20.8 μ, with 1–10 longitudinal and 5–13 transverse septations; beaks straight, slender, short to long attenuate, quite variable in length, hyaline, occasionally with enlarged basal cells, smooth, 2–6-septate, 20–336 × 1.3–5.0 μ, averaging 106–121 × 2.2–2.4 μ; in culture conidia occasionally formed in 2-spore chains. Parasitic on foliage of *Cucumis melo* L., *C. sativus* L., *Citrullus vulgaris* Schrad., *Cucurbita pepo* L., *C. maxima* Duchesne, and *C. moschata* Duchesne.

SUMMARY

Alternaria cucumerina was found to be the cause of a leafspot disease of cucurbits in all cases examined during the past 2 years in Florida. A study of this species indicates that the size range of conidia is sufficient to include *A. brassicae* f. *nigrescens* Pegl. Accordingly, *A. brassicae* f. *nigrescens* and a derived combination, *A. nigrescens* (Pegl.) Neerg., are regarded as synonyms. An emendation to the present descriptions of the fungus is proposed.

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LITERATURE CITED

1. **Boughey, A. S.** 1946. A preliminary list of plant diseases in the Anglo-Egyptian Sudan. Imp. Mycol. Inst., Mycol. Paper 14. 16 pp.
2. **Elliot, J. A.** 1917. Taxonomic characters of the genera *Alternaria* and *Macrosporium*. Am. Jour. Bot. **4**: 439–475.
3. **Ellis, J. B., and B. M. Everhart.** 1895. New species of fungi from various localities. Proc. Acad. Nat. Sci. Phila. **1895**: 413–441.
4. **Jackson, C. R.** 1958. Taxonomy and host range of *Alternaria cucumerina*. (Abs.) Phytopathology **48**: 343–344.
5. **Lanjouw, J., et al.** 1956. International code of botanical nomenclature. Kemink en Zoon, N.V., Utrecht, Holland. 340 pp.
6. **Nattrass, R. M.** 1932. Annual report of the mycologist for 1931. (Abs.) Rev. Appl. Mycol. **11**: 695–696.
7. **Neergaard, P.** 1945. Danish species of *Alternaria* and *Stemphylium*. Oxford Univ. Press, Oxford. 560 pp.
8. **Nicolas, G.** 1934. Sur un *Alternaria* parasite du melon. Rev. Pathol. Veg. Entomol. Agr. France **21**: 15–17.
9. **Peglion, V.** 1893. Una nuova malattia del melone cagionata dall' *Alternaria Brassicae* f. *nigrescens*. Riv. patol. veg. **1**: 296–299.
10. ——. 1893. Sopra due parassiti del melone. Note di patalogia vegetale. Riv. patol. veg. **2**: 227–240.
11. ——. 1895. Contribuzione alla conoscenza della flora micologica avellinese. Malphigia **8**: 424–460.
12. **Saccardo, P. A.** 1913. Sylloge fungorum **22**: 1410.
13. **Schwarze, C. A.** 1917. The parasitic fungi of New Jersey. New Jersey Agr. Exp. Sta. Bull. 313. 226 pp.

THE ASCOCARPS OF ASPERGILLUS ALLIACEUS

DOROTHY I. FENNELL AND J. H. WARCUP

(WITH 11 FIGURES)

Aspergillus alliaceus, described in 1926 by Thom and Church (**4**) as a new sclerotial species in the *A. wentii* Group, was based upon two strains: C.T. 4660, isolated by Eddy from a decaying garlic bulb sometime prior to 1924 when it was discussed without name by Walker and Lindegren (**6**) as a wound parasite of onions; and C.T. 4656, isolated from a dead blister beetle (*Macrobasis albida*) by Cohen and High of the Bureau of Chemistry and submitted to Thom for identification in 1922. Thom and Church recognized that *A. alliaceus*, as exemplified by these strains, might develop a perfect stage, and in their original description reported unsuccessful attempts "to induce the development of an ascogenous phase in these sclerotia whose appearance is so suggestive of perithecia."

The discovery by Raper, Fennell, and Tresner (**3**) of an ascosporic stage in the sclerotioid parenchymatous structures of *Aspergillus citrisporus* and *A. ornatus*, members of the "sclerotial" *A. tamarii* section of the genus, further suggested that the development of a perfect stage by other sclerotium-producing species of *Aspergillus* could be anticipated. Although careful examination of sclerotia from numerous cultures of the *A. wentii* and *A. flavus* Groups over a period of many years had failed to disclose a single ascosporic strain, these authors recognized the possibility of their existence and concluded that "extension of our search for additional forms, and the introduction of new procedures for isolating them, may in time justify our optimistic hope."

During an investigation of the fungous flora of Australian soils by one of us (J. H. W.), many sclerotial species of *Aspergillus* were isolated. All such isolates were held for extended periods and, following publication of the description of *A. ornatus*, these sclerotial forms were re-examined for the presence of a perfect stage. The sclerotia of two isolates, SA 15 and SA 117, in cultures about a year old were discovered to be full of spores. In order to determine their nature, new cultures were prepared and examined at regular intervals; after three months,

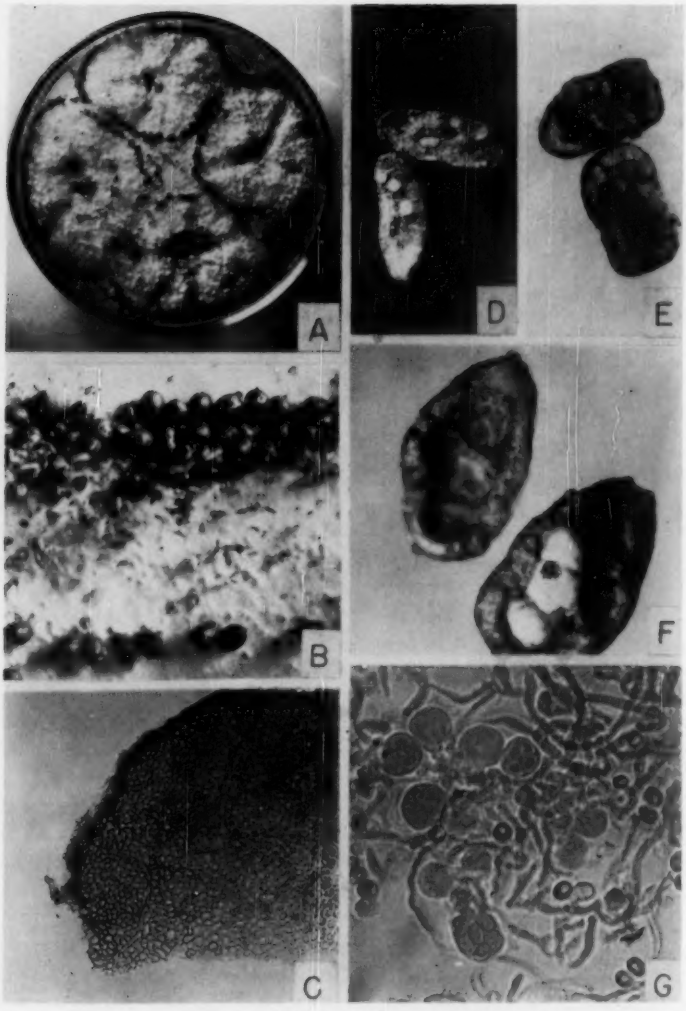

FIG. 1, A–G.

FIG. 1. *Aspergillus alliaceus* Thom and Church. A. Colonies developed from single ascospores of QM 1892, steep agar, 10 days. B. Stromatic bodies of QM 1892, × 5. C. Hand-section of stromatic body of QM 1892, showing internal sclerenchymatous tissue as it appears just prior to the appearance of recognizable

asci and ascospores were seen in SA 117 and somewhat later in SA 15. The latter strain, although it continues to produce abundant sclerotia, has failed to develop ascospores in more recent tests.

The two isolates, SA 15 and SA 117, were forwarded in September 1954, to K. B. Raper, University of Wisconsin, for examination and for his opinion regarding the possibility that they represented a new species. Raper immediately recognized their cultural similarity to *Aspergillus alliaceus*, and microscopic examination of the conidial structures verified this relationship. However, the isolates from Australia consistently produced larger and more elongate sclerotia than those of the type strains studied by Thom and Church (**4**).

The two ascosporic strains from Australia were then sent to the Quartermaster Laboratories, where they were grown in comparative culture with one of the co-types of *Aspergillus alliaceus* (C.T. 4656; QM 1885). Once again SA 117 produced ascospores at three months and SA 15 failed to do so at any age up to two years. The representative strain of *A. alliaceus*, examined at lengthening intervals, finally was observed to produce the ascigerous stage at ten months, although it had been regarded as a strictly sclerotial culture for thirty years. Cleistothecia, asci, and ascospores of the Thom strain and of those from Australia are identical.

The discovery of a delayed ascogenous stage in the presumed "sclerotia" of *Aspergillus alliaceus* necessitates an enlargement of the original diagnosis and the following emended description is proposed:

ASPERGILLUS ALLIACEUS Thom and Church emend.

Colonies on Czapek's-solution agar growing rapidly, 6.0–7.0 cm in ten days at 25° C, composed of a compact, white basal felt with loosely floccose aerial mycelium about 1 mm deep, developing abundant silvery gray to black "sclerotia" in more or less concentric zones, producing a limited number of upright conidiophores bearing radiate conidial heads which rarely affect the colony appearance; no characteristic odor noted; exudate fairly abundant, clear, collecting in droplets on the sclerotia, upon evaporation leaving slight depressions or circular areas of slightly different color (FIG. 1, B); reverse white to cream to yellow with the black sclerotia easily visible through the mycelium. Conidial heads

cleistothecia, × 110. D. Stromatic body from QM 1892 laid open to show four randomly situated immature cleistothecia, × 7.5. E. Stromatic body from QM 1885 showing a single cleistothecium, × 25. F. Stromatic body from QM 1892 containing three mature cleistothecia, somewhat shrunken during photographing. Their imprint is shown plainly in the empty half on the left, × 25. G. Fertile hyphae, asci, and ascospores of QM 1892, × 560.

radiate, splitting into divergent columns in age, at first yellow, becoming dull golden brown to buff, variable in size, 100–1000 μ in diameter, smaller heads borne on short conidiophores from aerial mycelium, larger structures on long conidiophores from substrate hyphae; conidiophores smooth, yellow, sinuous, variable in size, 0.2–3 mm × 7.5–15.0 μ, with walls up to 2.0 μ thick; vesicles globose, thick-walled and showing

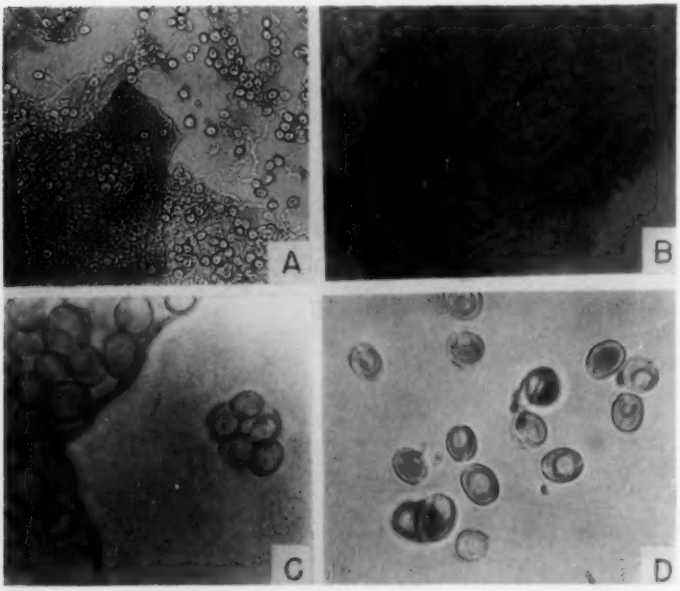

FIG. 2. *Aspergillus alliaceus* Thom and Church. A. Fragment of cleistothecial wall, × 245. B. As A, in greater detail, showing flattened, irregular, elongate cells composing the cleistothecial walls, × 1000. C. A single ascus and ascospores visible through the extremely thin wall of a completely mature cleistothecium, from QM 1885, × 1025. D. Mature ascospores of QM 1885, showing eccentric oil droplets and equatorial lines, × 1025.

pores after the sterigmata are detached, fertile over their entire surface, variable in size, 15–75 μ in diameter but mostly 50–60 μ; sterigmata in two series, extremely crowded, primaries 7–13 × 3–5 μ, each bearing 6 to 8 secondary sterigmata; secondaries mostly 9.0–10.0 × 1.5–2.2 μ but varying from 7.0–13.0 μ in length, with sides usually parallel but sometimes inflated with age; conidia smooth-walled, yellow, oval to subglobose, 3.0–4.0 × 2.5–3.5 μ. Sclerotia erect, sessile or borne on a suggestion of a stalk, horny, ovate to elliptical, 1–3 mm in vertical axis by

500–700 μ in diameter, at first white, then gray-black and white-tipped, finally becoming black in age, composed of greenish, thick-walled, sclerenchymatous tissue throughout (FIG. 1, C). Initiation of the ascogenous phase has not been observed but may be evidenced by the "channelling" of the stromatic tissue (FIG. 1, C) which precedes the appearance of independent ascocarps at one to seven or eight loci randomly scattered throughout the tissue (FIG. 1, D and E), the number of these locules appearing to be directly correlated with the size of the stromatic body. Cleistothecia (FIG. 1, F) globose, variable in size, consisting of ascogenous hyphae, asci, and ascospores (FIG. 1, G) surrounded by thin sheath-like membranes composed of single layers of irregular, flattened cells (FIG. 2, A and B); maturing extremely slowly, requiring from three to ten months depending upon the strain; at maturity completely filling the stromatic body except for a tough outer wall usually 150–200 μ thick (FIG. 1, F). In age the thin walls of the separate cleistothecia rupture and the entire cavity is filled with free ascospores; viewed at this stage the stromatic bodies appear to be single heavy-walled ascocarps. Asci (FIGS. 1, G; 2, C) 8-spored, oval to globose, 15–18 μ in diameter, evanescent after maturation of the ascospores; ascospores (FIG. 2, C and D) uncolored, smooth, thin-walled, elliptical, variable in size, mostly 5.5–9.0 × 5.0–7.0 μ, with younger spores showing a fine equatorial furrow that becomes less evident in age; mature spores containing an eccentric oil droplet. Ascospore germination commences in the equatorial region but in most instances is preceded by simple swelling rather than by the valvular separation that is characteristic of ascospore germination in other ascigerous *Aspergilli*. Colonies developing from single ascospores (FIG. 1, A) are entirely typical of the parent strains.

Cultures examined:

SA 15 (QM 1891)—Isolated by J. H. Warcup, 1952, from soil (red-brown earth), Waite Institute, Adelaide, S. Australia.

SA 117 (QM 1892)—Isolated by J. H. Warcup from a sandy loam soil from Mt. Riddock, central Australia.

SA 122—Source as SA 15.

C.T. 4656 (QM 1885)—Isolated by Cohen and High from a dead blister beetle (*Macrobasis albida*) at the Pharmacognosy Laboratory of the Bureau of Chemistry, Washington, D. C.

DISCUSSION

In the belief that the members of the genus *Aspergillus* (including those species which develop a perfect stage) form a natural and cohesive entity, Thom and Raper (**5**) recognized three ascosporic sections: the *A. glaucus,* the *A. fumigatus,* and the *A. nidulans* Groups; and, as in

earlier publications, they reduced to synonomy other names of generic rank which had been used for these groups. With the subsequent description of the ascigerous stages of *A. ornatus* and *A. citrisporus* (**3**), the *A. tamarii* section was recognized as a fourth ascosporic group. More recently, Benjamin (**1**) has advocated the re-establishment of the genera *Eurotium*, *Sartorya*, and *Emericella* to cover the first three of these ascosporic sections but has failed to provide a suitable disposition of *A. ornatus* and *A. citrisporus*. The ascosporic stage of *A. alliaceus*, described in the present paper, differs so markedly from that known in any other *Aspergillus* that its placement in one of the previously known ascigerous sections of the genus is untenable. It is likewise impossible to place this species in either *Eurotium*, *Sartorya*, or *Emericella* and, if one followed this system, it would be necessary to establish not only a fourth genus to cover *A. alliaceus* but also a fifth to provide for *A. ornatus* and *A. citrisporus*. Since we believe, with Thom and Raper (**5**) and Raper (**2**), that such fragmentation of the genus *Aspergillus* is undesirable, we prefer simply to recognize that a fifth major section, the *Aspergillus wentii* Group, has been added to the list of ascigerous *Aspergilli*.

In all of the ascosporic species thus far recognized in *Aspergillus* the cleistothecia show a marked degree of similarity in the structure of their walls, which at maturity consist of a thin (usually single) layer of flattened cells. In members of the *A. glaucus* Group these cells generally are unobscured by enveloping hyphae and the yellow cleistothecia appear naked. In *A. fischeri* the ascocarp initially appears to have a cottony consistency and one gains the impression of a truly fibrous outer wall; at maturity, however, most of this outer covering can be removed, revealing the thin wall of cells to which a portion of the enveloping hyphae still may be attached. In the *Aspergillus nidulans* Group the cleistothecial wall appears somewhat fibrous in the early stages but at maturity consists of a thin layer of flatened angular cells. Cleistothecia in this group are characterized particularly by a loose envelope of thick-walled hülle cells. In *A. ornatus* the cleistothecium initially suggests a developing sclerotium and when broken open is seen to consist of a mass of angular parenchymatous cells. With further development, cells in the central area of the ascocarp are digested by the developing ascogenous tissue and finally a thin outer layer of flattened polygonal cells remains. In *A. alliaceus* the multiple cleistothecia develop within a persistent, indurate mass of sclerenchymatous tissue. Considered as individual structures, however, the ascocarps are not basically different from those in the groups previously studied. Here also the outer covering of a

mature cleistothecium consists of a thin layer of irregularly elongate, flattened cells.

SUMMARY

The description of *Aspergillus alliaceus* Thom and Church, a member of the *A. wentii* Group, is emended to include a perfect stage which differs from all other ascosporic *Aspergilli*. Multiple discrete thin-walled cleistothecia develop within a sclerotioid stromatic body which retains a heavy outer wall even when the ascocarps are completely mature.

ACKNOWLEDGMENT

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LITERATURE CITED

1. **Benjamin, C. R.** 1955. Ascocarps of *Aspergillus* and *Penicillium*. Mycologia **47**: 669–687.
2. **Raper, K. B.** 1957. Nomenclature in *Aspergillus* and *Penicillium*. Mycologia **49**: 644–662.
3. **Raper, K. B., D. I. Fennell, and H. D. Tresner.** 1953. The ascosporic stage of *Aspergillus citrisporus* and related forms. Mycologia **45**: 671–692.
4. **Thom, C., and M. B. Church.** 1926. The Aspergilli. Williams and Wilkins Co., Baltimore, Md. *272* pp.
5. **Thom, C., and K. B. Raper.** 1945. A Manual of the Aspergilli. Williams and Wilkins Co., Baltimore, Md. *373* pp.
6. **Walker, J. C., and C. C. Lindegren.** 1924. Further studies on the relation of onion scale pigmentation to disease resistance. Jour. Agr. Res. **29**: 507–514.

SPERMATIUM–TRIGHOGYNE RELATIONSHIP IN GELASINOSPORA CALOSPORA VAR. AUTOSTEIRA [1]

ROGER D. GOOS [2]

(WITH 8 FIGURES)

Although experimental work has shown that spermatia are effective as fertilizing agents in a number of Ascomycetes (Dodge, **1932**; Ames, **1932**; Drayton, **1932**; Dimock, **1937**; Groves and Drayton, **1937**; and Zickler, **1934, 1937**), the mechanism by which fusion between the spermatium and the receptive hyphae is effected has been shown in relatively few genera. Higgins (**1936**) illustrated the long, tapering trichogynes of *Mycosphaerella tulipifera* with attached spermatia, and described the migration of the spermatial nucleus into the ascogonium. Ames (**1934**) demonstrated the probable method of plasmogamy in *Pleurage anserina*, and Zickler (**1937, 1953**) has described the process, including migration of the spermatial nucleus into the ascogonium, in considerable detail for *Bombardia lunata*. Shoemaker (**1955**) has reported observing enucleate spermatia attached to trichogynes within six hours after spermatization in *Cochliobolus sativus*.

Sloan and Wilson (**1958**) have recently demonstrated that the spermatia produced by *Gelasinospora calospora* (Mouton) Moreau and Moreau var. *autosteira* (Alexopoulos and Sun) Alexopoulos and Sun are capable of acting as fertilizing agents. This fungus, originally described by Alexopoulos and Sun (**1950**), is heterothallic and mycelia derived from single ascospores belong to one of two compatibility groups. To obtain perithecia, either compatible mycelia must be mated, or the protoperithecia of a receptive strain must be spermatized with compatible spermatia. Tylutki (**1958**) demonstrated that the compatibility factor is controlled by a single pair of alleles which segregate in a 1:1 ratio at meiosis; he also demonstrated that mycelia derived from single

[1] Portion of a thesis submitted to the Graduate College of the State University of Iowa in partial fulfillment of the requirements for the degree of Doctor of Philosophy, under the direction of Professor C. J. Alexopoulos.

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ascospores are essentially hermaphroditic, since they possess the capability of producing both spermatia and protoperithecia.

Both Tylutki (**1958**) and Sloan and Wilson (**1958**) suggested the probability of a spermatium-trichogyne relationship in *G. calospora* var. *autosteira*. However, inasmuch as none of these investigators had demonstrated the mechanism by which plasmogamy is effected in this fungus, it was thought desirable to initiate studies in this regard.

MATERIALS AND METHODS

Cultures used for this study were descendants of the original strains of *G. calospora* var. *autosteira* isolated by Alexopoulos and Sun (**1950**), and were obtained by mating strains used by Sloan and Wilson (**1958**), and germinating the resulting ascospores. Strains designated G-1 and G-3 were chosen for this study, since they produce numerous perithecia when crossed, and the G-1 strain produces large numbers of spermatia. Both strains, however, produce spermatia and protoperithecia.

In all spermatization experiments the G-3 strain served as the receptive strain. Reciprocal crosses (i.e. G-3 spermatia on G-1 protoperithecia) were successful, but fewer perithecia resulted since the G-1 strain produces fewer protoperithecia.

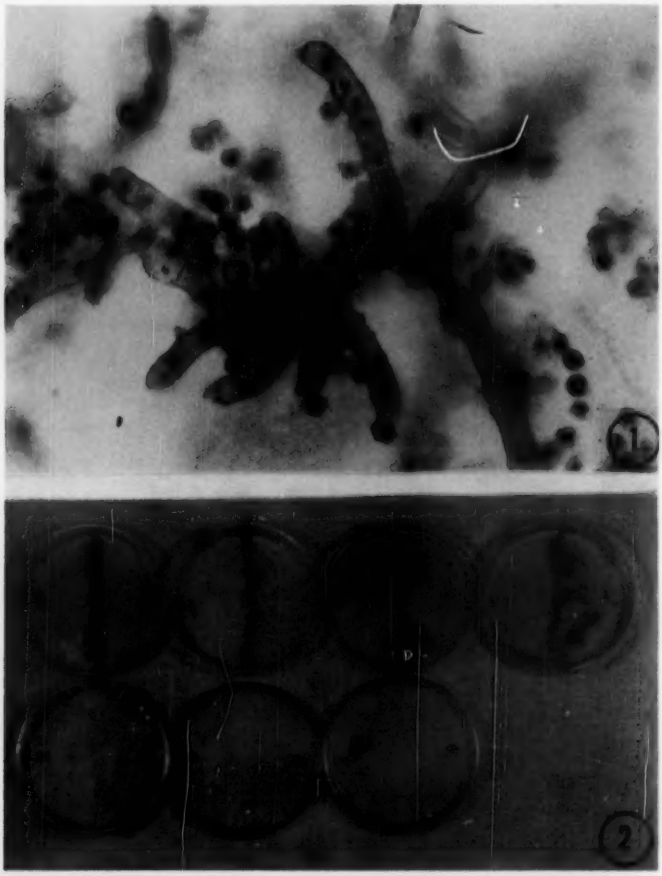
All cultures were grown at temperatures of 20° C under direct light, as suggested by Tylutki (**1958**). Temperature was controlled by placing the cultures in a Labline #3552 Ambi-Lo temperature control cabinet. Light was supplied by a 15 watt Ken-rad fluorescent bulb suspended 4½ inches above each shelf of the cabinet. Light intensity varied from 100 to 300 foot candles at different points on the same shelf, depending on the distance from the bulb. Difco corn meal agar, prepared according to the manufacturer's directions and supplemented with 1 g each of Difco malt extract and Difco yeast extract per liter, was used as the medium throughout the investigation.

Ascospore germination was induced by placing ascospores on malt-peptone agar (1.5% Difco malt extract, 0.2% Bacto-peptone) containing 1 part per 100,000 furfural, as recommended by Emerson (**1948**). The spores were then slowly heated to 60° over a period of 30 minutes, after the method of Dodge (**1912**).

Spermatial suspensions were prepared according to the methods outlined by Sloan and Wilson (**1958**). Spermatization was carried out by placing a few drops of the spermatial suspension (0.4 ml) within a sterile glass ring placed on the culture surface. Under the culture conditions employed, five-day-old cultures were found to be highly

receptive to spermatia, and spermatizations were normally carried out with cultures of this age.

Observations of plasmogamy were made on both living and fixed material, although, as will be discussed later, little success was attained



FIGS. 1–2. *Gelasinospora calospora* var. *autosteira*. 1. Spermatiophores and spermatia stained with Giemsa stain. Note the large prominent nucleus in each cell of the spermatiophore; also the phialide-like projection from which spermatia are abstricted. × 1300. 2. Results obtained when the mycelia derived from the seven viable spores of a single ascus were paired with the protoperithecial parent.

with living material. Temporary mounts were prepared by removing a block of material from a culture, trimming away excess agar so that a thin layer of the culture surface remained, fixing and staining. Mounts in which nuclei could be observed were obtained with both the propionocarmine and Giemsa stains, following fixation over 1% osmium tetroxide. The Giemsa stain gave especially good nuclear differentiation when the material was treated according to the following schedule: fix over the vapors of 1% osmium tetroxide for one to one and one-half minutes. Add a few drops of distilled water to the mount, and then add one to two drops of the undiluted stain. Allow a few seconds for the stain to penetrate, then wash off excess stain by flooding with water. Clear and differentiate by adding one or two drops of 3% KOH, wash with water, and observe.

RESULTS AND OBSERVATIONS

Spermatia and Spermatiophores—Tylutki (**1958**) was the first investigator to report the presence of spermatia and spermatiophores in *G. calospora* var. *autosteira*. The spermatiophores are branched structures, well differentiated from somatic hyphae, and appear similar in their morphology to those reported for *G. adjuncta* by Cain (**1950**). The branches consist of short cells, each of which is essentially a spermogonium and each of which bears a small, phialide-like projection from which spermatia are abstricted. In preparations stained with Giemsa stain, the nuclear condition of the spermatiophore could be observed. Each short cell of the spermatiophore branches was found to contain a single large nucleus (FIG. 1). The spermatium likewise appears to be uninucleate; however, since these small cells generally take a dense stain, it is difficult to be certain of their nuclear condition. Their small size (2–3 μ), however, and the presence of a single nucleus in the spermogonium, supports the belief that the spermatia are uninucleate.

Observations on Plasmogamy—In attempts to follow the process of plasmogamy, development of the protoperithecium was studied to determine, if possible, (1) whether trichogynes are present, (2) if they are present, from what structures they develop, and (3) the earliest point in the development of the protoperithecium at which they could be distinguished. Such studies were made on agar-coated cover slips, using both mated and unmated cultures.

The protoperithecium is initiated as a lateral hyphal branch which almost immediately begins to coil, soon forming a tight helical coil. Soon after this stage of development, hyphae are produced at the base of the coil, which grow up to envelop and enclose the ascogonial coil.

Subsequent development of the ascogonium is obscured in living material by the enveloping layer. The coil may be seen, however, in young protoperithecia stained with aceto-carmine, cotton blue, or Giemsa stain, indicating that it remains as a differentiated structure. The enveloping layer soon becomes pseudoparenchymatous in nature. Numerous hyphae begin to develop from the young protoperithecium, and extend in all directions from it. Development ceases at approximately this stage, unless the protoperithecium is fertilized.

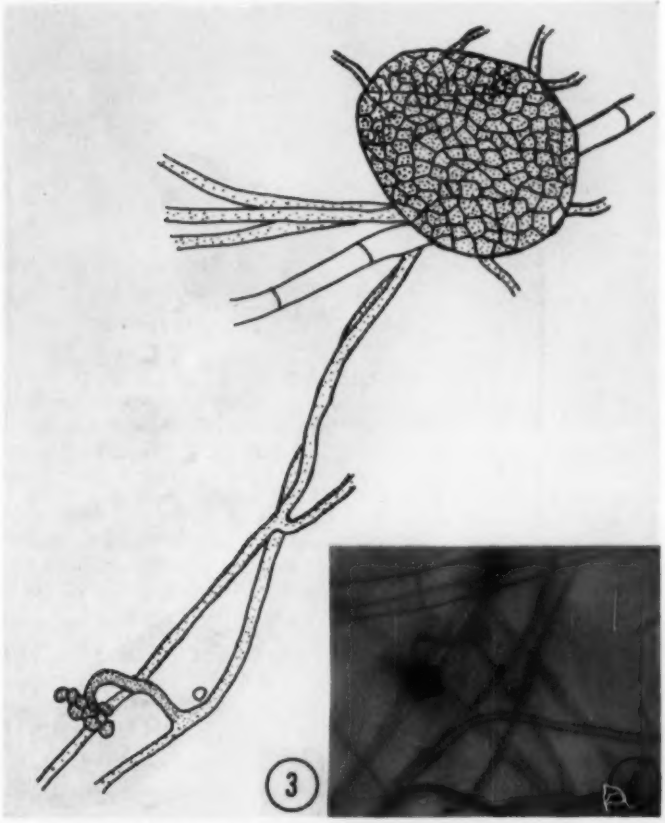
An examination of microtome sections shows that nearly all of the hyphae extending from the protoperithecium originate in the outer enveloping layers. The ascogonial coil can be distinguished in such sections as a very densely staining structure within the pseudoparenchymatous mass. A few sections were found in which extensions from the ascogonial coil could be followed to the exterior of the protoperithecium. It is believed that these extensions develop into trichogynes, and thus connect the ascogonial coil with the exterior of the protoperithecium. Because of the abundance of sterile hyphae which develop from the pseudoparenchymatous walls of the mature protoperithecium, it is not possible to identify the trichogynes with certainty, even in stained mounts. Tylutki (**1958**) distinguished structures he interpreted to be trichogynes in very young protoperithecia, by their differential staining, but he emphasized that they could be observed only in the very early stages of development. He likewise found that he could not distinguish such structures in mature protoperithecia. The observations made in the present study are thus in agreement with those of Tylutki.

Since it is not possible to distinguish the receptive hyphae in mature photoperithecia with certainty, attempts to observe their response to the presence of compatible spermatia are not possible. However, by examining stained mounts of spermatized areas at various intervals following spermatization, it is possible to determine what is taking place. Mounts from spermatized areas were made at intervals varying from four to twenty-four hours after spermatization. Inasmuch as the receptive hyphae are difficult to recognize, search was made for the presence of hyphae which were in contact, or possibly fusing with, spermatia.

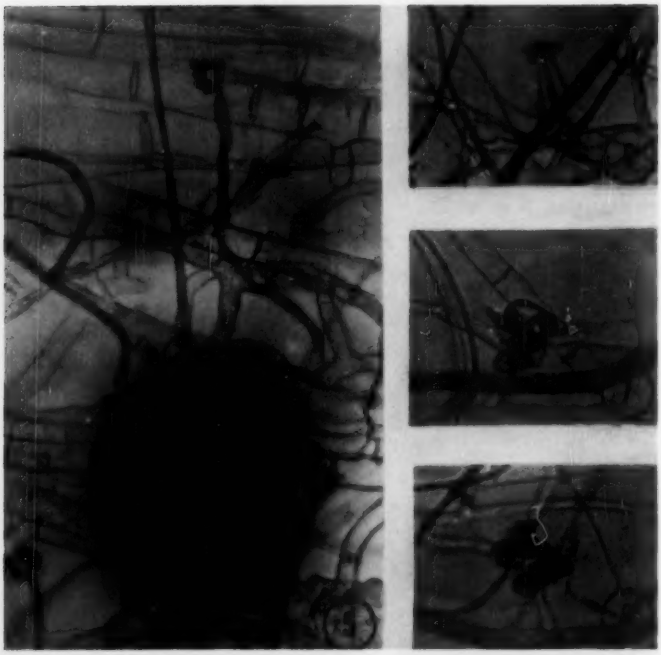
The first evidence of plasmogamy was found in mounts prepared seven and one-half hours after spermatization (FIGS. 3, 4, and 5). In these mounts, certain hyphae were found which showed a distinct predilection for the spermatia. These hyphae could be readily distinguished, inasmuch as they stained very densely. These hyphae were in intimate contact with spermatia and in certain instances appeared to have fused with spermatia. Some of them could be traced to protoperithecia,

whereas others, because of their length and the abundance of somatic hyphae around them, could not.

In mounts prepared eleven hours after spermatization, similar densely staining hyphae were found directed toward groups of spermatia. In one instance, a single spermatial cluster was found with three densely stained hyphae directed toward it. In many of the mounts prepared eleven hours after spermatization, the tips of the trichogynes were found



FIGS. 3–4. *Gelasinospora calospora* var. *autosteira*. 3. Camera lucida drawing of a protoperithecium with a trichogyne extending to a group of spermatia, from a mount prepared 7½ hours after spermatization. × 200. 4. Photograph of the tip of the trichogyne illustrated in Fig. 3.



FIGS. 5–8. *Gelasinospora calospora* var. *autosteira*. 5. Protoperithecium (out of focus) with a trichogyne in contact with a spermatium, seen in a mount prepared 7½ hours after spermatization. × 200. 6. Trichogyne tip fused to a spermatium, from a mount prepared 11 hours after spermatization. × 900. 7. Tip of a trichogyne coiling among a group of spermatia, found in a mount prepared 11 hours after spermatization. × approximately 900. 8. Trichogyne tips found in contact with spermatia, found in a mount prepared 11 hours after spermatization. × approximately 900.

to have branched and coiled among the spermatia (FIGS. 6, 7, and 8), thus establishing intimate contact with the spermatia. In most instances these densely staining hyphae could not be traced from the spermatial groups to protoperithecia, because of the large number of somatic hyphae present in such mounts. A sufficient number could be traced, however, so as to leave little doubt that all those observed originated from protoperithecia, or branched from hyphae which did so.

A degenerative process appeared to be occurring in some of the densely staining hyphae in mounts prepared fourteen hours after spermatization. The cytoplasm, especially in the portions nearest to the

spermatia, appeared coagulated, and large vacuoles were present. This was observed in several different instances, and was interpreted to be an indication that the spermatial nucleus had passed through the trichogyne. Because of the coiling of the hyphal tips in the vicinity of the spermatia, it was difficult to determine the nuclear condition of the spermatia in contact with such trichogynes. However, some spermatia which appeared empty were found attached to hyphae whose protoplasm appeared to be degenerating.

In mounts prepared twenty-four hours after spermatization, hyphae completely devoid of protoplasmic contents could be seen extending from protoperithecia into groups of spermatia. In manner of branching and growth, they resembled the densely staining hyphae found in preparations made soon after spermatization. It could not be definitely established that these hyphae had fused with spermatia, inasmuch as rather large groups of spermatia were present and obscured vision; but the observations made in the earlier preparations lead one to believe that they had.

The densely staining hyphae found in the mounts described above were interpreted to be trichogynes. Their manner of staining, and their apparent response to the presence of spermatia, indicate that they are specialized hyphae. Their dense staining indicates that they were in a highly active state at the time of fixation. The trichogynes, as distinguished in such mounts, may be quite extensive. They may branch soon after leaving the protoperithecium, and may rebranch. Trichogynes extending at least 1 mm from the protoperithecium were observed, and presumably longer trichogynes may occur. The trichogynes appeared to have made a tropic response to the presence of spermatia. Such a response was not observed in living trichogynes, but in fixed material the trichogynes appeared to have grown toward spermatial groups (FIGS. 3 and 4), some having made a nearly right-angle change in direction of growth. This response appears to be confined to the tip of the trichogyne, or to the tip of one of its lateral branches. Nuclear migration from a spermatium into a trichogyne was not confirmed by cytological studies, but evidence was obtained to show that it does occur.

Conclusive proof that the spermatial nucleus takes part in karyogamy and contributes genetic material to the fusion nucleus can be obtained, if it can be shown that a genetic factor controlled by the spermatial parent can be recovered from the ascospores produced by a spermatized culture. Tyiuitki (**1958**) has shown that the mating-type factor is controlled by a single pair of alleles that segregate in a 1:1 ratio at meiosis;

hence, this is a convenient factor to study. If, of the eight spores present in a single ascus, four are of mating type *A* and the remaining four are of mating type *a*, proof that the spermatial nucleus is involved in fertilization will have been obtained.

Accordingly, asci were removed from perithecia produced by a spermatized culture and the ascospores isolated. The spores from six asci, from each of which five or more viable spores were isolated, were analyzed for mating type by pairing the mycelia derived from single spores with the G-3 parent. The results are shown in TABLE I, and are illustrated in FIG. 2. It is readily evident that spores of both mating types were present in each ascus analyzed, and that they occurred in as near a 1:1 ratio as it was possible to obtain with the number of viable

TABLE I

ANALYSIS OF THE MATING TYPES OF SPORES IN SINGLE ASCI PRODUCED BY SPERMATIZED CULTURES

Ascus	Number of viable spores isolated	Mycelia of mating type A	Mycelia of mating type a
1	5	2	3
2	7	3	4
3	8	5*	3
4	6	4	2
5	6	2	4
6	7	4	3

Mycelia derived from single ascospores were mated with the protoperithecial parent to determine mating type.

* An extraneous spore was probably included among these spore isolates.

spores isolated per ascus. From these results there can be no doubt that the spermatial nucleus is included at karyogamy and that genetic material is contributed.

Somatogamy—It is of interest to determine whether plasmogamy is limited to a spermatium-trichogyne relationship, or whether it might also occur through somatic fusions. Sloan and Wilson (**1958**) attempted to resolve this point, and concluded that somatogamy does not readily occur in this fungus, and that compatible nuclei occur in close association only in the ascogonium and ascogenous hyphae. These conclusions were based on hyphal-tip studies, in which hyphal tips growing out from the base of mature perithecia, or from agar blocks removed from areas where compatible mycelia met in mated cultures, were isolated and placed in single-tip culture. In over two hundred such isolations, no perithecium-producing cultures were found, indicating that none of them were heterokaryotic for the mating type factor. During

the present study, over one hundred hyphal tips were removed from hyphae that grew out from the base of mature perithecia produced by a mated culture. None of the cultures thus obtained produced perithecia, although mycelia of both mating types were obtained.

Sloan and Wilson (**1958**) also used U-tubes to study the possibilities of somatic fusions. They found that when the two arms of a U-tube were inoculated with opposite strains, the mycelia met between the arms of the tube, but perithecium production occurred in only one of 43 U-tube cultures.

In the present study, modified growth tubes were used to study the possibility of somatogamy. These tubes were eight to nine inches long, and were depressed on the upper side for a distance of about one cm. at the midpoint, closing approximately one-half the diameter of the tube. Such tubes were filled with agar medium to the level of the depression. This allowed two air chambers to be formed in each tube, but permitted the medium to form a continuous layer. When the tubes were inoculated at opposite ends with compatible strains, the aerial portions of the two strains were separated by the glass depression, but the mycelia were free to intermingle within the medium. When these tubes were inoculated on one end with a single strain, the fungus soon grew throughout the full length of the tube. When inoculated with compatible strains, the mycelia of the two strains met beneath the depression and intermingled. It was possible to determine with a binocular dissecting microscope that hyphae of both strains were present; however, no evidence of fusion could be seen. Although numerous protoperithecia developed on the agar surface, many of them adjacent to the glass depression, none of them developed into perithecia, even after six weeks of incubation. Perithecia developed only after the formation of an air space, and were then restricted to the point where the two mycelia met. These results indicate that compatible nuclei are not readily brought into intimate association by the fusion of the somatic hyphae, and suggest that plasmogamy may be limited to a spermatium-trichogyne relationship.

DISCUSSION

The results of the present investigation indicate that a spermatium-trichogyne relationship exists in *G. calospora* var. *autosteira*. The trichogyne is not a well-differentiated structure in this species, and is difficult to recognize, even in stained preparations, because of the numerous outgrowths from the mature protoperithecium. This is in contrast to the situation as reported by Backus (**1939**) for *Neurospora*,

in which the trichogynes took a deep stain, even with such general stains as lacto-phenol with cotton blue. The trichogynes of *Gelasinospora* stained densely only when they were found in the vicinity of spermatia. However, since these hyphae established intimate contact with spermatia and appeared to have fused with them, and since they could be traced from groups of spermatia to protoperithecia, there is little doubt that they are the receptive hyphae. It is not known how many trichogynes arise from a single protoperithecium. Protoperithecia with more than one receptive hypha in contact with spermatia were never encountered, although two branches from a single trichogyne system were found to be in contact with spermatia.

Many of the reports concerning spermatium-producing Ascomycetes and lichens contain statements to the effect that trichogynes appear to be attracted to spermatia. This is reported by Bachmann (**1910**) for the lichen genus *Collema*. Backus (**1934**) reported a tendency of the trichogynes of *Coccomyces hiemalis* to grow towards the microconidium-producing areas, although he was unable to establish that these cells function in plasmogamy in this species. Zickler (**1953**) reported that the trichogyne of *Bombardia lunata* will grow in a random manner for some distance, but when it happens into the area of a spermatium or spermogonium, the tip grows toward this fertilizing element, sometimes making a right angle turn. He also found that trichogynes would respond to capillary tubes containing filtrates from spermatial suspensions. Bistis (**1957**) has found that directional growth of trichogynes occurs in *Ascobolus stercorarius*, the trichogyne growing toward a compatible oidium, if the oidium is within a radius of 100 μ.

The observations made in the present investigation indicate that the trichogynes of *G. calospora* var. *autosteira* also show tropic responses to the presence of spermatia. Such reactions have not been observed in living material, but have been inferred from the manner of growth observed in slide preparations. It appears likely that plasmogamy in this species is not a passive process, restricted to those occasions where spermatia happen to come into contact with a trichogyne, but that the trichogynes may actually reach the spermatia by growth of their apical regions.

Recovery of both mating types from perithecia produced by spermatized cultures offers proof that the spermatial nucleus is involved in karyogamy. Moreau and Moruzi (**1931**) considered perithecium production in *Neurospora sitophila* to be solely the result of hormonal stimulation. Dodge (**1932**) and Aronescu (**1933**) could find no evidence to support Moreau and Moruzi's claim. Aronescu (**1934**) removed and grew ascospores from single asci, and in every case found

a one-to-one segregation of the two mating types. Recovery of the two mating types from the spores of a single ascus indicates that nuclei of both mating types are involved in karyogamy. Additional evidence that perithecium production in *Gelasinospora* is dependent on more than hormonal stimulation is offered by the fact that perithecia were never produced in the modified-growth-tube experiments, even though compatible mycelia were grown in a common medium, with only the aerial portions of the mycelia separated.

SUMMARY

The role of spermatia in the process of plasmogamy in the heterothallic fungus *Gelasinospora calospora* var. *autosteira* has been studied. From observations made on material killed at various intervals following spermatization, it is concluded that this fungus produces trichogynes, which appear to grow toward the spermatia, presumably through a tropic response. Trichogynes were found to fuse with spermatia. The trichogynes were found to form branched systems, extending 1 mm or more from the protoperithecium. The receptive region appears to be near the tip of the trichogyne. Migration of nuclei from spermatia into trichogynes was not confirmed by cytological study, but mycelia derived from ascospores removed from single asci were of both mating types. Segregation of mating types occurred in each ascus in as near a 1:1 ratio as it was possible to obtain with the number of viable spores isolated. It is thus possible to conclude that the spermatial nucleus is involved in karyogamy and contributes genetic material.

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LITERATURE CITED

1. **Alexopoulos, C. J., and S. H. Sun.** 1950. A new species of *Gelasinospora*. Mycologia **42**: 723–734.
2. **Ames, L. M.** 1932. An hermaphroditic self-sterile but cross fertile condition in *Pleurage anserina*. Bull. Torrey Bot. Club **59**: 341–345.
3. ——. 1934. Hermaphroditism involving self-sterility and cross fertility in the Ascomycete *Pleurage anserina*. Mycologia **26**: 392–414.

4. **Aronescu, A.** 1933. Further studies in *Neurospora sitophila*. Mycologia **25**: 43–54.
5. ——. 1934. Further tests for hormone action in *Neurospora*. Mycologia **26**: 244–252.
6. **Bachmann, F. M.** 1912. A new type of spermogonium and fertilization in *Collema*. Ann. Bot. **26**: 747–760.
7. **Backus, M. P.** 1934. Initiation of the ascocarp and associated phenomena in *Coccomyces hiemalis*. Contr. Boyce Thompson Inst. **6**: 339–379.
8. ——. 1939. The mechanics of conidial fertilization in *Neurospora sitophila*. Bull. Torrey Bot. Club **66**: 63–76.
9. **Bistis, G.** 1957. Sexuality in *Ascobolus stercorarius*. II. Preliminary experiments on various aspects of the sexual process. Am. Jour. Bot. **44**: 436–443.
10. **Cain, R. F.** 1950. Studies of coprophilous Ascomycetes. I. *Gelasinospora*. Canad. Jour. Res. C. **28**: 566–576.
11. **Dimock, A. W.** 1937. Observations on sexual relations in *Hypomyces ipomoeae*. Mycologia **29**: 116–127.
12. **Dodge, B. O.** 1912. Method of culture and the morphology of the archicarp in certain species of the Ascobolaceae. Bull. Torrey Bot. Club **39**: 139–197.
13. ——. 1932. Heterothallism and hypothetical hormones in *Neurospora*. Bull. Torrey Bot. Club **58**: 517–522.
14. ——. 1932. The non-sexual and sexual functions of microconidia of *Neurospora*. Bull. Torrey Bot. Club **59**: 347–360.
15. **Drayton, F. L.** 1932. The sexual function of the microconidia in certain Discomycetes. Mycologia **24**: 345–348.
16. **Emerson, R. M.** 1948. Effect of furfural on germination of *Neurospora* spores. Jour. Bact. **55**: 327–330.
17. **Groves, J. M., and F. L. Drayton.** 1939. The perfect stage of *Botrytis cinerea*. Mycologia **31**: 485–489.
18. **Higgins, B. B.** 1936. Morphology and life history of some Ascomycetes with special reference to the function of spermatia. Am. Jour. Bot. **23**: 598–602.
19. **Moreau, F., et C. Moruzi.** 1931. Recherches expérimentales sur la formation des perithèces chez les *Neurospora*. Compt. Rend. Acad. Sci. Paris **192**: 1476–1478.
20. **Shoemaker, R. A.** 1955. Biology, cytology, and taxonomy of *Cochliobolus sativus*. Canad. Jour. Bot. **33**: 562–576.
21. **Sloan, B. J., and G. B. Wilson.** 1958. The function of the microspores of *Gelasinospora calospora* var. *autosteira*. Mycologia **50**: 111–116.
22. **Tylutki, E. E.** 1958. Some aspects of morphology, genetics, and cultural behavior of *Gelasinospora calospora* var. *autosteira*. Mycologia **50**: 333–356.
23. **Zickler, H.** 1934. Genetische Untersuchungen an einem heterothallischen Askomyzeten (*Bombardia lunata* nov. spec.). Planta **22**: 573–613.
24. ——. 1937. Die Spermatienbefruchtung bei *Bombardia lunata*. Ber. Deutsch. Bot. Ges. **55**: 114–119.
25. ——. 1953. Zur Entwicklungsgegeschicte des Askomyzeten *Bombardia lunata* Zckl. Arch. Protistenk. **98**: 1–70.

ON THE FUNGAL FLORA OF IRAQI SOILS. II. CENTRAL IRAQ

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(WITH 2 FIGURES)

Since the beginning of this century, the fungus flora of the soil has attracted the attention of various investigators, particularly in Europe and North America. In Iraq, with huge areas of cultivated land, a knowledge of the soil flora would hold much interest. There have apparently been no previous investigations of this type in Iraq. However, many other such studies were carried out in other countries, as those of Jensen (**6**), Waksman (**15**), Paine (**10**), Tresner et al. (**14**), and Miller et al. (**8**) in the United States of America, Bisby et al. (**2, 3**) in Canada, Warcup (**17**), Stenton (**12**) and Nicholls (**9**) in the British Islands, Jensen (**7**) in Denmark, Chaudhuri and Sachar (**4**) in India, Sabet (**11**) in Egypt, and Farrow (**5**) in Panama and Costa Rica. These reports have provided lists of fungi occurring under diverse environments.

The work here described was part of that planned by Tolba et al. (**13**) to constitute a survey of soil fungal flora of Iraq, which was started by a study of the fungal flora of Baghdad area (**13**). The present work was planned to study the soil fungal flora of Central Iraq.

MATERIALS AND METHODS

Soil samples were collected from five Liwaas (provinces) of Republic of Iraq. Samples were collected from one town in each Liwaa (FIG. 1) according to the following scheme:

Liwaa	Town
Baghdad	Mahmudiya
Hilla	Hilla
Karbala	Kufa
Dulaim	Falluja
Diala	Baquba

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The samples were collected from the surface layers (5 cm) with sterilized spoons, the material of each sample being mixed thoroughly and kept in sterilized bottles for further use.

The methods used for a) making isolations, b) determination of soil-water contents, c) determination of soil reaction, d) determination of

FIG. 1. Map of Iraq, showing the Liwaas (provinces) from which soil samples were collected.

total soluble salt contents of the soil, e) determination of the chloride contents of the soil, and f) estimation of organic matters in the soils were those mentioned in a previous paper (**13**).

Air-temperature, soil-temperature at a depth of 0.2 m, rainfall, and percentage relative humidity were kindly supplied by the Iraqi Meteorological Department. The average values of these records are plotted graphically in FIG. 2, which shows that Central Iraq is characterized by a dry hot summer and a rainy cold winter.

DISCUSSION OF THE RESULTS

The results in TABLE I show that the frequency of occurrence of fungi differs markedly in the various soil samples collected. Thus, the highest number of fungal colonies was isolated from soils collected from Mahmudiya and Kufa and the lowest came from Falluja and Hilla,

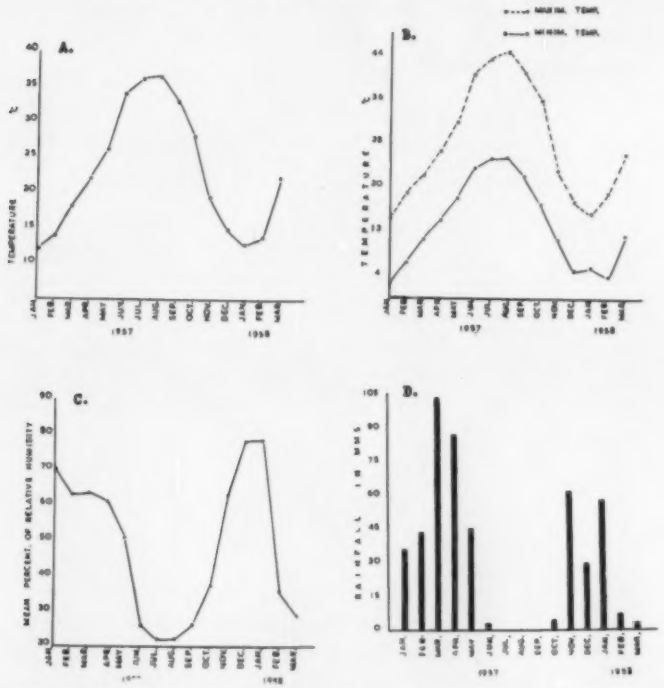

FIG. 2. Monthly averages of: A. Soil temperature (in degrees Centigrade) at a depth of 0.2 m; B. Air temperature; C. Percentage relative humidity; and D. Total monthly rainfall (in mm) during the years 1957 and 1958 in the various provinces of central Iraq.

while count of fungi in Baquba soil occupied an intermediate position between these two extremes. Examination of the total soluble salt-contents of the various soil samples (TABLE I) revealed a strong negative correlation between fungal and total soluble salt contents. Thus the low fungal population in Hilla and Falluja soils is easily explicable on the basis of the high soluble salt contents of the soils which, presum-

ably, check the growth of the fungi inhabiting such soils. The reverse statements hold true for the soil samples collected from Mahmudiya and Kufa. Similar correlations between salt contents and number of fungi in the soil were reported earlier by Sabet (**11**) in Egyptian soils and by Tolba et al. (**13**) in Iraqi soils.

The results in TABLE I also support the view expressed earlier by Tolba et al. (**13**) that the frequency of occurrence of fungal isolates in the soil is much more influenced by the salt-content of the soil than by its content of organic matter.

TABLE I

DISTRIBUTION OF FUNGI IN SOILS COLLECTED FROM DIFFERENT PROVINCES IN CENTRAL IRAQ IN RELATION TO SOIL REACTION, SOIL MOISTURE, ORGANIC MATTER, TOTAL SOLUBLE SALTS, CHLORIDE CONTENTS, AND WATER-HOLDING CAPACITY OF THE SOIL

Item	Locality from which soil sample was collected				
	Mahmudiya	Falluja	Baquba	Kufa	Hilla
No. of fungal colonies/g dry soil	282,000	29,000	100,000	227,000	27,000
Organic matter content: %	2.87	2.77	2.55	3.70	2.82
Total soluble salts: %	0.15	0.86	0.45	0.33	1.23
Chlorides: %	0.021	0.014	0.007	0.005	0.182
pH-value	6.7	7.1	6.7	7.2	7.3
Water-holding capacity: %	55.7	46.8	44.0	63.6	56.3
Water-content: % w.h.c.:					
1/11/1957	8.1	42.3	5.2	28.6	5.3
6/12/1957	37.5	35.7	44.3	40.7	40.3
18/1/1958	52.1	65.8	75.0	53.5	46.5
13/2/1958	21.6	37.2	42.7	26.6	25.2
21/3/1958	3.9	38.2	10.9	35.2	27.4

TABLE II gives the relative percentage distribution of the main genera of fungi isolated from soils collected from the five provinces of central Iraq. Each fungal isolate was counted, in the original counts, as one whether it occurred singly or in a mixture with other fungi on the isolation plate.

The results in TABLE II show that:

a) In considering all samples the order of occurrence for the chief genera was *Hormodendrum, Aspergillus, Fusarium, Alternaria, Penicillium, Humicola, Mucor,* and *Pythium.* This is very much different from the order of soil fungi reported by American investigators for soils of various American States. Thus Abbott (**1**) in Louisiana and Werkenthin (**19**) in Texas both found *Aspergillus* dominant. Waksman

(15) in New Jersey lists *Penicillium, Mucor, Aspergillus, Trichoderma, Cladosporium, Fusarium,* etc. in that order. Bisby et al. (2, 3) for Manitoba found the order to be *Penicillium, Fusarium, Trichoderma, Rhizopus, Cladosporium,* and *Aspergillus.* Miller et al. (8) in Georgia reported the order to be *Penicillium, Aspergillus, Cunninghamella, Trichoderma, Rhizopus, Gliocladium* and *Fusarium.*

TABLE II

RELATIVE DISTRIBUTION OF THE MAIN GENERA OF FUNGI ISOLATED FROM SOILS COLLECTED FROM DIFFERENT PROVINCES IN CENTRAL IRAQ.

(Expressed as percentage of isolations)

Fungus	Locality from which soil was collected				
	Mahmudiya	Falluja	Baquba	Kufa	Hilla
Alternaria	16.45	1.75	—	12.20	13.85
Aspergillus	26.61	66.50	11.81	5.60	8.90
Bartalinia	6.16	—	—	0.20	—
Cephalosporium	1.22	—	1.00	2.20	0.20
Fusarium	2.00	2.45	43.30	3.50	1.75
Gliocladium	0.07	—	0.70	1.00	—
Helminthosporium	0.07	—	—	0.20	1.45
Hormiscium	—	0.35	0.15	3.60	5.00
Hormodendrum	17.36	8.75	4.20	54.10	54.75
Humicola	0.07	—	16.10	—	1.10
Mucor	2.16	8.40	4.30	1.30	0.35
Oospora	—	—	1.05	—	—
Penicillium	16.11	1.75	3.00	8.80	0.70
Phoma	—	—	3.80	—	—
Pythium	0.88	5.98	2.62	1.90	2.50
Rhizopus	0.81	—	2.50	0.60	1.75
Spicaria	4.54	—	0.15	—	5.70
Stemphylium	1.15	0.70	0.15	0.07	—
Sterile nonsclerotial mycelium	—	1.40	—	2.40	—

The high and low frequencies of occurrence of *Aspergillus* and *Penicillium* respectively in the soils being examined are, most probably, due to the hot climate prevailing in the country, particularly during the months from April to September. It may be recalled here that similar conclusions were suggested earlier by Werkenthin (19), Waksman (16), Jensen (7), Sabet (11), and Tolba et al. (13) with respect to American, Egyptian and Iraqi soils.

b) In the various soil samples considered separately the order of dominance shows great variation; thus:

1. *Aspergillus* constituted the fungal population in the soil collected from Falluja.

2. *Aspergillus, Hormodendrum,* and *Penicillium* were the main genera isolated from Mahmudiya soil.

3. *Fusarium* was dominant in the soil collected from Baquba. This is followed by *Humicola* and *Aspergillus.*

4. Species of the genus *Hormodendrum* constituted the greatest part of the fungal populations of Kufa and Hilla soils.

No regular correlation can, however, be observed between the order of dominance of fungi in the various soil samples and any of the following soil factors: organic matter content, total soluble salts content, chloride content, pH value, water-holding capacity or water content. Furthermore, no appreciable differences were observed in the various meteorological factors in the different localities from which the samples were collected to account for the varied orders of dominance of fungi observed.

It thus appears that the order of dominance of fungi in the different soil samples is governed by factors other than those previously mentioned. It is most likely, therefore, that such order is appreciably affected by the nature of the components of the soil.

The species of fungi isolated from the various soil samples being tested are set out in TABLE III which shows that the total number of species isolated was 150, belonging to 41 genera. Most of those enumerated are copious spore-bearers and it is possible that they have a numerical advantage because of the methods used in this investigation.

The number of species of fungi isolated was approximately the same as or slightly greater than that found by Jensen (**6**), Abbott (**1**), Bisby et al. (**2, 3**), Farrow (**5**), Warcup (**18**), Tolba et al. (**13**), Miller et al. (**8**) and others; and so it is possible that most of the soil-inhabiting fungi, except of course the Basidiomycetes and possibly some other mycelial forms, were discovered.

The number of species in each of the four classes of fungi is: Phycomycetes, *26*; Ascomycetes, 5; Basidiomycetes, none; Fungi Imperfecti, 119; total, 150. In common with the findings of other soil-fungus studies, the majority of species belong to the Fungi Imperfecti. Phycomycetes were mainly represented by fungi belonging to the genera *Mucor, Rhizopus* and *Pythium.* Members of the Ascomycets were of very rare occurrence (14 out of approximately 3800 isolates) on the isolation plates. Mycelia Sterilia were not uncommon. *Rhizoctonia solani* was met with in a very few cases on the isolation plates, but brown, grey, and white types of septate mycelia which formed no sclerotia were met with on the isolation plates when inoculated with soils collected from Falluja and Kufa.

TABLE III

LIST OF GENERA AND SPECIES OF FUNGI ISOLATED FROM FIVE PROVINCES IN CENTRAL IRAQ

Fungus	Locality from which soil was collected				
	Baquba	Falluja	Hilla	Kufa	Mahmudiya
Acrothecium apicale Berkeley & Broome	−	−	−	−	+
Acrothecium robustum Gilman & Abbott	−	−	−	−	+
Alternaria fasiculata Cooke & Ellis	−	−	+	+	+
Alternaria geophila Daszewska	−	+	−	+	+
Alternaria grisea Szilvinyi	−	−	+	−	+
Alternaria humicola Oudemans	−	+	−	+	+
Alternaria tenuis Nees	−	+	+	+	−
Alternaria sp.	−	−	−	−	+
Aspergillus flavus Link	+	+	+	+	−
Aspergillus fumigatus Fresenius	+	−	−	+	+
Aspergillus Koningi Oudemans	−	−	−	−	+
Aspergillus luchuensis Gnui	−	−	−	+	+
Aspergillus niger Van Tieghem	+	+	+	+	+
Aspergillus ochraceous Wilhelm	−	−	−	−	+
Aspergillus panamensis Raper & Thom	−	−	+	−	+
Aspergillus sparsus Raper & Thom	+	−	−	−	−
Aspergillus tamarii Kita	+	+	−	+	−
Aspergillus terreus Thom	−	+	+	+	+
Aspergillus ustus (Bainier) Thom & Church	−	−	−	+	−
Aspergillus wentii Wehmer	+	−	−	−	−
Bartalinia sp.	−	−	−	−	+
Cephalosporium acremonium Corda	−	−	+	−	−
Cephalosporium asperum Marchal	−	−	−	−	+
Cephalosporium curtipes Saccardo	+	−	−	+	−
Cephalosporium roseo-griseum Saksena	−	−	−	+	+
Chaetomella sp.	+	−	−	−	−
Chaetomium trilaterale Chivers	+	−	−	−	−
Chaetomium sp.	−	−	−	−	+
Cunninghamella bertholletiae Stadel	−	−	−	−	+
Cunninghamella echinulata Thaxter	−	−	−	−	+
Cunninghamella sp.	−	−	−	−	+
Curvularia lunata (Walker) Boedijn	−	−	+	+	−
Curvularia pallescens Boedijn	−	−	−	+	−
Curvularia tetramera (McKinney) Boedijn	−	−	−	+	−
Fumago sp.	−	+	−	−	−
Fusarium angustum Sherbakoff	+	+	−	−	−
Fusarium bostrycoides Wollenweber & Reinking	+	−	−	−	−
Fusarium chlamydosporum Wollenweber & Reinking	+	−	−	−	−
Fusarium coeruleum (Libert) Saccardo	+	−	−	−	−
Fusarium conglutinans Wollenweber	+	−	−	−	+
Fusarium dimerum Penzig	+	−	−	−	−
Fusarium equiseti (Corda) Saccardo	+	−	−	−	−
Fusarium lactis Pirotto & Riboni	−	−	+	−	−
Fusarium lini Bolley	+	−	−	−	−
Fusarium merismoides Corda	+	−	−	−	−

+ = Fungus present.
− = Fungus absent.

TABLE III (*Continued*)

Fungus	Locality from which soil was collected				
	Buquba	Falluja	Hilla	Kufa	Mahmudiya
Fusarium moniforme var. *minus* Wollenweber	+	+	+	+	+
Fusarium neoceras Wollenweber & Reinking	+	−	−	−	−
Fusarium nivale (Fries) Cesati	−	−	−	+	−
Fusarium orthoceras Appel & Wollenweber	+	−	−	−	+
Fusarium poae (Peck) Wollenweber	+	+	−	+	+
Fusarium redolens Wollenweber	+	−	−	−	−
Fusarium sambucinum Fuckel	+	−	−	−	−
Fusarium sporotrichioides Sherbakoff	+	−	−	−	+
Fusarium trichothecioides Wollenweber	+	−	−	−	−
Fusarium vasinfectum var. *zonatum* (Sherbakoff) Wollenweber	+	−	−	−	−
Gelasinospora cerealis Dowding	+	−	−	−	−
Gliocladium catenulatum Gilman & Abbott	+	−	−	−	−
Gliocladium fimbriatum Gilman & Abbott	−	−	−	+	−
Gliocladium penicilloides Corda	−	−	−	+	−
Gliocladium roseum (Link) Thom	−	−	−	−	+
Gymnoascus subumbrinus A. L. Smith & Ramsbottom	+	−	−	−	−
Haplographium bicolor Grove	−	−	−	−	+
Helminthosporium anomalum Gilman & Abbott	−	−	+	−	+
Helminthosporium nodulosum (Berkeley & Curtis) Saccardo	−	−	+	+	−
Helminthosporium sativum Pammel, King & Bakke	−	−	+	−	−
Hormiscium stilbosporum (Corda) Saccardo	+	+	+	+	−
Hormodendrum cladosporioides (Fresenius) Saccardo	+	+	−	+	+
Hormodendrum hordei Bruhne	+	+	−	−	+
Hormodendrum nigrescens Paine	−	+	+	+	−
Hormodendrum olivaceum (Corda) Bonorden	−	+	−	−	−
Hormodendrum pallidum Oudmans	−	+	−	−	−
Hormodendrum resinae Lindau	−	+	+	+	−
Hormodendrum viride (Fresenius) Saccardo	−	+	+	+	−
Humicola brevis (Gilman & Abbott) Gilman	+	−	−	−	−
Hyalopus sp.	−	−	−	−	+
Macrosporium cladosporioides Desmazières	+	−	−	+	−
Monilia brunnea Gilman & Abbott	−	−	−	−	+
Monilia humicola Oudmans	−	+	−	−	−
Mucor abundans Povah	+	−	−	−	−
Mucor christianiensis Hagem	+	−	−	−	−
Mucor circinelloides van Tieghem	+	−	−	−	−
Mucor fragilis Bainier	+	+	−	+	−
Mucor globosus Fischer	+	−	−	−	−
Mucor griseo-cyanus Hagem	−	+	−	−	+
Mucor hiemalis Wehmer	+	+	−	−	−
Mucor luteus Linnemann	−	+	−	−	−
Mucor racemosus Fresenius	+	−	−	+	+

TABLE III (*Continued*)

Fungus	Locality from which soil was collected				
	Baquba	Falluja	Hilla	Kufa	Mahmudiya
Mucor silvaticus Hagem	−	−	+	−	−
Mucor varians Povah	+	+	−	−	−
Oospora variabilis (Lindner) Lindau	+	−	−	−	−
Penicilliopsis sp.	−	−	−	−	+
Penicillium baarnense van Bemya	−	+	−	−	−
Penicillium brevi-compactum Dierckx	+	−	−	−	+
Penicillium canadense A. Smith	−	+	−	+	−
Penicillium canescens Sopp	−	−	−	−	+
Penicillium chrysogenum Thom	−	−	+	−	−
Penicillium commune Thom	−	−	−	+	−
Penicillium coryophilum Dierckx	−	−	−	−	+
Penicillium daleae Zaleski	−	−	−	−	+
Penicillium decumbens Thom	+	−	−	+	−
Penicillium duclauxi Delacroix	−	−	−	−	+
Penicillium funiculosum Thom	−	+	−	−	−
Penicillium godlewskii Zaleski	+	−	−	−	−
Penicillium lanosum Westling	−	−	−	+	−
Penicillium namylowskii Zaleski	+	−	−	−	−
Penicillium notatum Westling	−	−	−	+	+
Penicillium oxalicum Thom	−	−	−	+	−
Penicillium palitans Westling	+	−	−	−	−
Penicillium roqueforti Thom	+	−	−	−	−
Penicillium rugulosum Thom	−	−	−	−	+
Penicillium steckii Zaleski	−	−	−	+	−
Pencillium stoloniferum Thom	−	−	+	−	+
Penicillium tardum Thom	−	+	−	−	−
Penicillium thomii Maire	−	−	−	+	−
Phoma hibernica Grims, O'Conner & Cummins	+	−	−	−	−
Pullularia pullulans (de Bary) Berkhout	−	+	−	−	−
Pythium aphanidermatum (Edson) Fitzpatrick	−	+	−	−	−
Pythium elongatum Mathews	−	−	−	−	+
Pythium intermedium de Bary	−	−	−	−	+
Pythium proliferum de Bary	−	+	−	−	−
Pythium pulchrum von Minden	−	+	−	−	+
Pythium rostratum Butler	−	+	+	−	+
Pythium ultimum Trow	+	+	+	+	+
Pythium vexans de Bary	−	+	−	+	−
Rhizoctonia solani Kuhn	+	+	−	+	−
Rhizopus arrhizus Fischer	+	−	−	+	−
Rhizopus cohnii Berlese & de Toni	+	−	−	−	−
Rhizopus nigricans Ehrenberg	+	−	−	+	−
Rhizopus oryzae Went & Gerlings	−	−	+	+	+
Sepedonium chrysospermum (Bulliard) Fries	−	−	−	+	−
Sepedonium sp.	+	−	−	−	−
Sphaeronaema sp.	+	−	−	−	−
Spicaria divaricata (Thom) Gilman & Abbott	−	−	+	−	−
Spicaria griseola Saccardo	−	−	−	−	+
Spicaria silvatica Oudemans	−	−	−	−	+
Spicaria simplicissima Oudemans	+	−	−	−	−
Spondylocladium australe Gilman & Abbott	−	−	−	+	+

TABLE III (*Continued*)

Fungus	Locality from which soil was collected				
	Baquba	Falluja	Hilla	Kufa	Mahmudiya
Spondylocladium xylogenum A. L. Smith	−	−	−	−	+
Sporotrichum pruinosum Gilman & Abbott	−	−	−	+	−
Stachybotrys atra Corda	−	−	−	+	−
Stemphylium botryosum Wallroth	+	−	−	−	−
Stemphylium macrosporoideum (Berkeley & Broome) Saccardo	−	+	−	−	+
Stemphylium verruculosum Zimmerman	−	−	−	+	+
Tetracoccosporium paxianum Szabo	+	−	−	−	+
Tetracoccosporium sp.	−	−	−	−	+
Tillachlidium humicola Oudemans	+	−	−	−	−
Tillachlidium sp.	−	−	−	−	+
Torula lucifuga Oudemans	+	−	−	−	−
Sterile non-sclerotial mycelium					
Brown	−	+	−	+	−
Grey	−	−	−	+	−
White	−	+	−	+	−

SUMMARY

1. Soils collected from five provinces of Central Iraq were examined for their fungal contents.

2. The frequency of occurrence of fungi differs markedly in the different soil samples.

3. Soluble soil contents seem to be more influential on the fungal population of a given soil than its organic-matter content.

4. One hundred and fifty fungal species, belonging to forty-one genera, were isolated from the different soils.

5. In common with other soil fungus studies, the majority of species isolated belonged to the Fungi Imperfecti.

6. In considering samples from all five provinces the order of occurrence of the chief genera of fungi isolated was *Hormodendrum, Aspergillus, Fusarium, Alternaria, Penicillium, Humicola, Mucor, Pythium.*

7. The order of dominance of the fungi shows great variation as among the various soil samples separately. No correlation could be observed between the order of dominance and the available data of the various soil and meteorological factors.

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LITERATURE CITED

1. **Abbott, E. V.** 1926. A study of microbiological activities in some Louisiana soils: a preliminary survey. La. State Uni. Exp. Sta. Bull. **194**: 1–25.

2. **Bisby, G. R., N. James, and M. Timonin.** 1933. Fungi isolated from Manitoba soil by the plate method. Canad. Jour. Res. **8**: 253–275.
3. **Bisby, G. R., M. Timonin, and N. James.** 1935. Fungi isolated from soil profiles in Manitoba. Canad. Jour. Res. C. **13**: 47–65.
4. **Chaudhuri, H., and G. S. Sachar.** 1934. A study of the fungus flora of the Punjab soils. Ann. Mycol. **32**: 90–100.
5. **Farrow, W. M.** 1954. Tropical soil fungi. Mycologia **46**: 632–646.
6. **Jensen, C. N.** 1912. Fungous flora of the soil. Cornell Agr. Exp. Sta. Bull. **315**: 414–504.
7. **Jensen, H. L.** 1931. The fungus flora of the soil. Soil Sci. **31**: 123–158.
8. **Miller, J. H., J. E. Giddens, and A. A. Foster.** 1957. A survey of the fungi of forest and cultivated soils of Georgia. Mycologia **49**: 779–808.
9. **Nicholls, V. O.** 1956. Fungi of chalk soils. Brit. Mycol. Soc. Trans. **39**: 233–238.
10. **Paine, F. S.** 1927. Studies of the fungous flora of virgin soils. Mycologia **19**: 248–267.
11. **Sabet, Y. S.** 1935. A preliminary study of the Egyptian soil fungi. Egypt. Uni. Bull. Fac. Sci. **5**: 1–29.
12. **Stenton, H.** 1953. The soil fungi of Wicken Fen. Brit. Mycol. Soc. Trans. **36**: 304–314.
13. **Tolba, M. K., Y. Al-Doory, and M. A. Al-Wahab.** 1957. On the fungal flora of Iraqi soils. I. Baghdad area. Proc. 3rd Arab Sci. Cong. (In press.)
14. **Tresner, H. D., M. P. Backus, and J. T. Curtis.** 1954. Soil microfungi in relation to the hardwood forest continum in southern Wisconsin. Mycologia **46**: 314–333.
15. **Waksman, S. A.** 1916. Soil fungi and their activities. Soil Sci. **2**: 103–155.
16. ——. 1917. Is there any fungous flora of the soil? Soil Sci. **3**: 565–589.
17. **Warcup, J. H.** 1951. The ecology of soil fungi. Brit. Mycol. Soc. Trans. **34**: 376–399.
18. ——. 1955. On the origin of colonies of fungi developing on soil dilution plates. Brit. Mycol. Soc. Trans. **38**: 298–301.
19. **Werkenthin, F. C.** 1916. Fungous flora of Texas soils. Phytopathology **6**: 241–253.

PLEOMORPHISM IN THE DERMATOPHYTES

G. N. Bistis

Pleomorphism in the dermatophytes has been the subject of numerous studies during the past century. In spite of this, progress towards an explanation of the phenomenon has been much slower than in other instances of instability in microorganisms. One of the principal reasons for the lag is that the more intensive work in the field was done during the early 1900's, before the introduction of the principles of microbial genetics prerequisite to a solution of the problem. There is the added difficulty of the lack of any recent critical attempt at a reexamination of these early findings. It is, consequently, the purpose here to correlate these results with the more recent work of pleomorphism, including my own observations, and to see whether such principles as mutation, selection, and norm of reaction can be used in a reexamination of the phenomenon. Since my experience has been limited to a study of one species, *Trichophyton mentagrophytes*, this paper will deal specifically with pleomorphism in this species and in general terms with pleomorphism in the dermatophytes as a whole.

The original biological material in this study was one strain of *T. mentagrophytes*, the *N* form, first described by Robbins (**1945**, **1949**) and kept in culture, apparently unchanged, up to the present time. It will be referred to here as the *N*, normal, or conidial form, to distinguish it from the pleomorphic variants derived from it. The nutrient medium used, a thiamine-neopeptone agar (McVeigh and Cambell, **1950**), was buffered with KH_2PO_4 (12.5 g/liter of medium). The incubation temperatures were 35° and 25° C, the latter also serving as temperature for storage of stock cultures. At 25° on this medium, the colonies of the *N* form produce large clumps of microconidia (en grappe) and are granular in appearance. In addition, chlamydospores, spirals and raquet hyphae are abundant. Macroconidia, on the other hand, are not normally found.

The term pleomorphism as applied to these fungi refers to the sudden appearance upon the surface of a colony of numerous white tufts of aerial hyphae projecting above the original mycelium. Under appropriate conditions these tufts increase in size and coalesce to form a single mass of mycelium overlying the original aerial system. In brief,

this is pleomorphism in the dermatophytes. With this description as a point of departure, the various investigators have attempted to answer one or more of the following basic questions: What are these tufts? Where do they originate? How do they originate, and how do they come to erupt over the surface of the colony? How many kinds are there? Why do they occur? When are they initiated? These basic questions, although stated so simply, have nonetheless proved to be extremely difficult and, even in the most favorable cases, have been only partially answered.

Of these questions, the first three form a natural group and will be discussed as a unit. What are these tufts? One of the original ideas pictured them as colonies of another organism existing as a commensal with the dermatophyte. This idea was abandoned when the patches were found to arise in uncontaminated colonies derived from single microconidia of the dermatophyte (Bodin, **1895**; Fox and Blaxall, **1896**). It was obvious that the tufts were derived from the dermatophyte itself. During this period (at the turn of the century) Sabouraud was completing an intensive study of this group of fungi, and in his book (Sabouraud, **1910**) there is a clear, concise presentation of his results as related to pleomorphism. My interpretation of these results is as follows:

In a fungus colony the juvenile cell form is that form exhibited by the cells at the peripheral growing region. In dermatophytes, as in most higher fungi, these cells are long, narrow, cylindrical-shaped segments of hyphae lying on or below the surface of the substrate. When such hyphal systems are traced back from the periphery towards the center of the colony, many of these cells and their descendants undergo a number of morphological modifications. These derived cell forms of the dermatophytes include chlamydospores and raquet hyphae that originate from the surface and subsurface hyphae primarily, and conidia, from the aerial mycelium. The conidia are of two types; unicellular microconidia and multicellular macroconidia; the latter are not always produced. When placed on fresh nutrient medium the various cell types send out branches or germ tubes whose segments are of the juvenile cell type, and the resultant colonies are identical with the colony from which the cells were taken. The term polymorphism has been applied to these cases of easily reversible modifications in cell morphology.

In contrast to the polymorphic modifications stand the alterations which lead to pleomorphism. They are irreversible in the sense that when mass transfers of hyphae are made from a pleomorphic tuft to fresh medium the resultant colony is morphologically different from the

parent colony on which the patch arose. Whereas the parent mycelium was granular in appearance and usually pigmented, the derived colonies are fluffy and generally non-pigmented. Microscopically the most striking feature is generally the absence of conidia in the derived colony. In a few instances, microconidia are found in greatly reduced numbers, but macroconidia are never in evidence. Most of the hyphal cells retain their juvenile morphology and, consequently, the polymorphic modifications are not as prevalent. The phenomenon of pleomorphism can then be considered as a transformation occurring in certain areas of a dermatophyte culture and confering upon the altered cells a greater tendency to retain the juvenile cell form. This tendency is reflected macroscopically by the fluffy aerial growth and microscopically by a paucity of conidia.

Despite considerable speculation, the nature of the change responsible for this transformation has never been clearly defined. Fox and Blaxall (**1896**) entertained the idea that it is a type of "sport." In recent years, following the application of the mutation theory to variation in fungi, the mutational origin of pleomorphism has been widely accepted (Sabouraud, **1929**; Emmons, **1932**; Gregory, **1935**; Wilhelm, **1947**; Robbins, **1949**). The term mutation as applied to pleomorphism must be used in its broadest sense, i.e., a heritable change resulting in a new phenotype, with no identification of the mutated entity.

But if mutation is the starting point, what is the relationship between this event and the macroscopic tuft of hyphae erupting over the surface of the colony? On this point our knowledge is scant; nevertheless, a working hypothesis, based upon certain reasonable assumptions and in harmony with the known facts, will be presented. The first assumption is that mutations occur sometime in their life history in a very small, randomly distributed portion of the cells constituting a colony. In some way each mutated unit is segregated into a hyphal system which grows and develops into a microcolony embedded in the meshes of the parent mycelium. The microcolony consists of radially oriented, growing, surface and subsurface hyphae and an aerial hyphal system, the tuft. The end product of one mutation is one pleomorphic patch.

The observations which led to the formulation of this hypothesis are as follows: Sabouraud noted that occasionally a patch may overtake the peripheral hyphae of the resident colony and become established in the agar. Robbins (personal communication) observed that following removal of the aerial mycelium of an *N* culture on which a number of tufts had appeared, the position of each tuft was clearly marked by an opaque, circular area in the agar. Microscopic examination revealed that the opaqueness was the result of hyphal proliferation; but lacking

an unequivocal morphological difference between normal and pleomorphic strands, the proliferating hyphae were not proved to be of the pleomorphic type.

Proof that the proliferating hyphae were pleomorphic was supplied by the following experiment performed by the author. Agar plates were inoculated with about 50 *N* microconidia evenly distributed over the surface of each plate. The growth within the colonies was of the usual density but very sparse in the interzones between colonies. Here, very few hyphae penetrated, and individual strands could be followed for some distance. In time, pleomorphic tufts appeared on the surface of some of these colonies and spread rapidly into the interzones. Within these zones numerous young, vigorously growing, radially oriented hyphal systems were found embedded in the agar, along with a few now partially lysed *N* strands. The orientation of the proliferating hyphae was such that they appeared to have their origin at the center of the pleomorphic tuft.

Although the growing hyphae were not organized into a dense frontier, in four instances single hyphal tips were obtained from them, and in each instance the resulting colony was of the pleomorphic type. Each patch, therefore, is composed not only of the very apparent aerial mass but also of a mat of subsurface hyphae and their aerial branches. The pleomorphic patch is, in effect, a colony growing within an established colony (the *N*). Although the pleomorphic and *N* hyphae are of the same species and the former must arise from a cell of the latter, there is no evidence of heterocaryosis. These four hyphal-tip strains have never thrown out sectors of normal growth. This homogeneity and stability holds for numerous single-hyphal-tip isolates obtained from aerial mycelium and indicates a gene mutation as the starting point. Furthermore, no hyphal fusions have ever been observed in our cultures, and when mixed inocula have been used—i.e., normal and pleomorphic—the resultant colonies were mixtures of pure normal and pure pleomorphic sectors.

In summary, at least a partial answer to the first three questions posed is now at hand. It is a reasonable assumption that a mutation, probably genic, in a single cell is the starting point for each patch. Separation of the mutated entity into a homocaryotic branch and the proliferation of this branch into an expanding microcolony results in the appearance of a macroscopic mass of aerial hyphae, the tuft. Growth is the mechanism by which these mutations come to macroscopic expression.

Although the tufts arising on the surface of a colony are all super-

ficially alike, Sabouraud's observations indicate that they may represent a variety of phenotypes. This leads to the next question that was asked: How many kinds are there? The papers of Catanei (**1929**) and Robbins (**1949**) firmly established the existence of intermediate forms. Robbins made mass transfers from a number of tufts and recorded the morphology of the resultant colonies. He found that they differed markedly in morphology, in contrast to the general similarity of the tufts from which they were derived. Some of the colonies were fluffy and produced few or no microconidia, whereas the majority were intermediates of various grades between the two arbitrary extremes represented by the conidial and the non-conidial, or mycelial, forms. Thus, even though the several tufts developing on the surface of a colony may be morphologically indistinguishable as tufts, when isolations are made to fresh medium the derived colonies reveal a marked variation.

Robbins also tested the relevance of the dual phenomenon which Wilhelm (**1947**) had applied to pleomorphism. Wilhelm postulated that the intermediates are heterocaryons carrying two types of nuclei, one determining the mycelial character, the other the conidial. An implicit assumption of this hypothesis is that gene mutation underlies the pleomorphic phenotype. Robbins isolated several single microconidia (unicellular, uninucleate spores) from each of a number of intermediates and found that the colonies which developed from these conidia were identical with the mother-colony types. The intermediates are homocaryotic and, consequently, the dual phenomenon does not apply to pleomorphism in this species. Rather, pleomorphism encompasses a graded series of mutants which have two characteristics in common. The first is curtailing of conidium production, and the second is an ability to grow within the meshes of an established *N* colony.

A subsidiary question arising from the results of Catanei and of Robbins is whether the transformation of the conidial to the highly mycelial form is a rigid step-by-step process with a number of prerequisite intermediate stages, or whether the complete transformation occurs in a single step—i.e., from conidial to mycelial. To answer this question, the following experiment was performed. Into a number of 9 cm Petri plates, 30 ml of the buffered thiamine-neopeptone agar was poured, allowed to solidify, and then inoculated over the entire surface with a 1 ml suspension of *N* microconidia. The plates were incubated at 35° and mass transfers made from several of the patches which appeared on each plate. In several instances, the mass inoculum taken from the first tuft to appear gave rise to a colony of the highly pleomorphic type, whereas many of the later-appearing tufts gave colonies

of some intermediate morphology. Consequently, if the assumption that each tuft is the product of a single mutational event is true, then it follows that the conidial form can change directly to the highly pleomorphic type. Furthermore, there is no apparent correlation between the time of appearance of a tuft and the degree of alteration shown by the colony derived from a mass transfer from the tuft. The further significance of this point will be made apparent shortly.

The last two fundamental questions to be considered are closely interrelated. Why do pleomorphisms occur, and when do they occur? For both questions, it is extremely important to specify in precise terms the nature of the subject. Does the term pleomorphism refer to the first mutated entity or to the end product, the tuft? One of the principal themes in this paper has been the distinction between the mutational event and the tuft but, unfortunately, a lack of appreciation of this distinction by many people in the field has led to serious errors in interpretation of data. It is now evident that one of the principle steps between the two, mutated entity and tuft, is governed by positive selection. The selective factor probably is some change in metabolism [1] which enables the mutant cells to grow in areas already occupied by an established *N* mycelium.

In the literature, the unwarranted assumption is quite often found that an external environmental condition which prevents the appearance of tufts is effective because it prevents the transformation. The possibility that transformation has occurred under these conditions and that negation of the positive selective factor is responsible for the absence of visible tufts was not considered. For example, in my work pleomorphic tufts have never been found in plates inoculated uniformly with a suspension of *N* conidia and incubated at 25°, whereas one or more tufts invariably appear in such plates incubated at 35°. The assumption, however, that the mutational event does not occur in the plates incubated at 25° is unwarranted. Some preliminary experiments indicate that the transformation occurs at 25°, but some factor operative at that temperature negates the selective advantage of the mutant cells; they do not grow, and consequently, no tufts appear.

One of the more prevalent notions is that cultivation on unfavorable media is the initiating factor, but, unfortunately, most of the proponents of this concept have failed to consider the distinction between initiation and the end product, the tuft.

[1] The work of Robbins and Ma (**1945**) and of McVeigh and Cambell (**1950**) indicates that the selective factor may be a change in nitrogen metabolism. They reported that the pleomorphic forms have a more effective mechanism for utilizing inorganic nitrogen.

For example, Grigoraki (**1925**), in an attempt to construct an integrated picture of pleomorphism and to apply it to the dermatophytes as a whole, embraced the concept of "progressive physiological degeneration" as a consequence of cultivation on unfavorable substrates. In essence, his concept of pleomorphism is as follows: When a dermatophyte is first isolated from its natural habitat, keratin structures of animals, macroconidia are the only spore type produced, and in this sense the fungus is monomorphic. Under the influence of these adverse conditions, there sets in a progressive step-wise degeneration involving a continual reduction in the number of macroconidia and the introduction of microconidial and chlamydospore production. At this stage the colonies are polymorphic in that more than one spore type is being produced. This irreversible degeneration continues: macroconidia are now absent, and microconidial and chlamydospore frequency drops until even these spore forms are no longer present. At this stage, the fungus is reduced to sterile mycelium consisting only of morphologically undifferentiated hyphae. This final phase of polymorphism is the completely degenerated or pleomorphic type. In Grigoraki's latest paper (**1956**), the hypothesis is restated in essentially its original form.

At the heart of the hypothesis are several concepts. First, the identification of the causative factor as unfavorable environment; secondly, that the degeneration is a continual irreversible progression; thirdly, that microconidia are a degenerate spore form; and finally, that polymorphism is an integral part of an irreversible degradation leading to the pleomorphic form.

Although no attempt will be made here to review the various papers which discuss the hypothesis, it is clear that it is not reconcilable with recent experimental results and must be rejected. Emmons (**1931**) was the first to test the validity of the hypothesis, and his was a simple and devastating experiment. He isolated single microconidia and macroconidia of a strain of *Achorion gypseum* (= *Microsporum gypseum*) and found that both types of spores yielded identical colonies with respect to conidium production. Thus, a supposedly degenerate microconidium was the progenitor of a colony which gave rise to a large number of non-degenerate macroconidia. Obviously, both spore types must have been at the same stage of degeneration, a conclusion inconsistent with the Grigoraki hypothesis. Further evidence against the hypothesis has already been presented in this paper. For example, the change occurs in highly localized areas and in many instances the first-appearing patch is of the highly pleomorphic type. The transformation then is not a continual general degeneration but rather one of series of

random changes which may occur in a small proportion of the cell population of the *N* colony.

Returning to the main topic, if the transformation is considered to be a mutation, the question now becomes, why does the mutation occur? Is it spontaneous or is it induced by mutagens produced by the fungus itself? For a population of cells as represented by a growing fungus colony there is as yet no satisfactory measure of mutation rate and consequently, no way of deciding between the two alternatives of spontaneous or auto-induced mutation. For pleomorphism, the only parameter available is the number of patches which come up over the surface of a colony. This number is generally 5–20 per 9 cm Petri dish. If it is assumed (1) that every patch arises from a single mutational event, (2) that all such events come to expression as patches, (3) that one locus is involved, then on an estimate of 10^8 nuclei per *N* colony a mutation rate of 10^{-7} would be required. This value is well within the frequency range of spontaneous mutants in other organisms.

Although absolute frequency may require nothing more than spontaneous mutation, a correlation between pleomorphism and age of the colony has been noted by a number of workers and the suggestion made that automutagenesis is the reason for the correlation. But again, this apparent relationship must be restated more precisely. It is clear that a pleomorphism-time relationship should not be based upon the time at which the tuft becomes a visible entity, but rather upon the age of the cell in which the mutational event itself has occurred. Only by indirect methods can any information be gotten as to the time of such an event. It was noted that the tufts first generally were seen about the sixth day after the first *N* hyphae had grown into a given area. This lag time also had a maximum of about 14 days so that if no tuft had appeared upon a given area within 14 days none would develop. These results indicated that mutation can occur in six-day-old, or even younger cells, but information on the length of the incubation period—i.e., the interval between initiation and expression as a visible tuft—was required.

By means of reconstruction experiments, which admittedly do not duplicate the conditions under which tufts normally arise in culture, single pleomorphic tips were placed at the periphery of *N* cultures and the time required for their development into visible patches recorded. The incubation period determined in this way ranged from six to nine days. Thus, a naturally occurring patch which is first observed on the sixth day after the first hyphae entered the area presumably had its origin as a mutation in very young cells just at their time of formation or

shortly thereafter. This still does not resolve the question of a pleomorphism-age correlation since the critical parameter is not the time at which a particular mutation occurs but, rather, the change in the probability of a mutation with increasing age of the cell or colony.

On the assumption that mutagens accumulate in aging cultures of *T. mentagrophytes*, several preliminary experiments were performed designed to test the effectiveness of staling products and intra-cellular sterile filtrates in increasing the frequency of mutation to pleomorphism in germinated conidia. The results were negative.

With the concepts of pleomorphism outlined in this paper as a foundation, its unusual features (Tate, **1929**) are easily reconciled with the assumption that pleomorphism is not essentially different from the usual run of variants found in other fungi. Tate outlined these features as: (1) Mutations in other fungi are usually reversible; pleomorphism is not. (2) Mutants are usually limited to certain areas of the culture, whereas pleomorphic tufts overgrow the entire surface of the original colony. (3) A given mutant appears rarely, whereas one or more pleomorphic patches are found in every colony. (4) Mutations are of various kinds. Pleomorphism is always associated with simplification of structure.

If the important characteristic, that pleomorphism confers upon the cell an ability to grow in areas already occupied by the resident colony, is taken into account, then the above differences are easily resolved. For example, point (2) can be resolved in the following way: A mutation occurring in the periphery of a growing colony, if it confers no new synthetic abilities upon the mutated cell, will generally manifest itself as a wedge-shaped sector with its apex at the point of origin of the first mutant cell. If such a mutation occurs back of the frontier, growth of the mutant sector would be expected to stop, in most cases, before it reached macroscopic dimensions. If, on the other hand, the mutation confers metabolic capacities which allow the mutant cell to grow within the meshes of the parent colony, then such a cell, whether it arises at the periphery of a colony or in a more central area, should give rise to a roughly circular patch of growth. If a number of such mutant areas occur within a colony, and if their growth potential is of sufficient magnitude, they should be able to overgrow the entire surface of the resident colony. As for point (3), spontaneous mutation plus positive selection could account for the appearance of tufts in every culture grown under conditions where such selection was operative (Brown, **1926**). Conversely, point (1) may be explained on the basis of extremely low back-mutation rate and negative selection. The summation of both factors could make detection of reversion to normal extremly unlikely. Point

(4) merely expresses the relationship between simplification of structure and the ability to proliferate in an area already occupied by the parent colony.

That mutations in other fungi are of various kinds—i.e., yielding a variety of phenotypes—is well established. Emmons (**1932**) reported that a variety of mutants other than the pleomorphic group can be obtained from old cultures of dermatophytes. The pleomorphic phenotype is the expression of only a sample of the possible mutations in these fungi; this sample consists of those mutants which combine the two characteristics simplicity of structure and increased synthetic ability.

The emphasis up to this point has centered about the multi-organism system represented by a normal colony and the pleomorphic microcolonies growing within its meshes. Now, I wish to compare and contrast this normal strain with the pleomorphic variants which can be isolated from it and cultured separately. The most striking morphological difference involves conidium production. These variants show great differences in microconidium production and range through a number of intermediates, which show consecutive stages in diminution of conidial number from the highly conidial normal form, to the highly pleomorphic type which produces few or no conidia.

Although for a given set of environmental conditions the variant strains fall into a distinct series, it is well known that striking differences in conidium production occur when a given strain is subjected to a variety of conditions (Davidson and Gregory, **1934**; Acton and Dey, **1934**; Stockdale, **1953**; Chin and Knight, **1957**). It has been my experience that differences in temperature, medium, desiccation, etc., profoundly affect both the number of microconidia produced and their spatial orientation (en grappe vs. en thyrse). Similar effects upon number of macroconidia were noted, and desiccation proved to be one of the most effective means for increasing their number. Indeed, most pleomorphic strains have been induced to produce large numbers of en-grappe microconidia and even macroconidia, thereby assuming the granular macroscopic aspect of the normal strain. Conversely, the normal strain when cultured on Sabouraud's agar at 35° will produce only a few small en-grappe clusters of microconidia and will take on the fluffy macroscopic appearance of a pleomorphic strain. Thus, phenotype can be altered either by some heritable change or by a change in environment, and the expressed phenotype is the outcome of the interaction of a given genotype with a given environment. In most instances, the heritable change does not result in an irrevocable loss of ability to form conidia but, rather, alters the norm of reaction. The differences

between strains reside in their patterns of reaction to external conditions and, consequently, a valid comparison is possible only when they are cultivated in the same environment. This author cannot accept the conclusion of Chin and Wright that such conversions from pleomorphic to granular gross morphology brought about by changes in environment constitute any sort of evidence against the mutation hypothesis of pleomorphism. These environmental effects are all easily reversible, whereas the inherited pattern of reaction of a particular strain changes only when the genotype changes.

The above account of environmental interaction holds for most isolates. There are several reports in the literature, however, which suggest that some strains have reached a point of no return. Langeron and Milochevitch (**1937**), in an attempt to obtain the stable reversions from pleomorphic to normal reported by Acton and Dey, found that certain of the highly pleomorphic strains were unaffected by the change in environment and did not produce greater numbers of conidia. Chin and Wright found one pleomorphic strain which would not respond to the CO_2 treatment effective in promoting conidium production in other pleomorphic strains. In our material, the normal and intermediate forms represent the group easily modified by environment and the highly mycelial represents the type which forms few microconidia under any circumstances. This confuses the issue as to what constitutes a pleomorphic strain. Should the original concept of a pleomorphism as any tuft of white down erupting on the surface of a colony be retained? Since our normal strain when grown on Sabouraud's agar at 35° has a pleomorphic gross morphology (fluffy, white) would some of the tufts sprouting on the surface of a more highly conidial strain grown under these conditions yield the normal strain? Such highly conidial strains do exist, the so-called granular type of Georg (**1956**). If so, then pleomorphic strains must be so designated with reference to some standard strain and set of conditions. For example, colonies of one intermediate strain, which grows as a granular colony at 25° on the thiamine-neopeptone agar, gave rise to pleomorphic patches when transferred daily to fresh medium (Bistis, **1956**). Or should the line of demarcation be drawn on the basis of susceptibility to external conditions with only the highly mycelial type considered as truly pleomorphic? Clearly, the problem has not yet been resolved.

Unfortunately, number of conidia and their spatial orientation are not characters which lend themselves to simple quantitative measurement. In an attempt to put the differences between the various strains on a more easily determined quantitative basis, growth curves have been

obtained for the normal, an intermediate, and a highly pleomorphic strain. The analysis of these growth curves will be presented in a subsequent paper.

In conclusion, the concept of pleomorphism in *Trichophyton mentagrophytes* as presented in this paper can be briefly outlined. In the life history of a colony of this species there occur mutations, but because of the absence of a sexual phase the nature of the mutated entities is unknown. Representatives of one family of mutants, the pleomorphic strains, under proper conditions appear as small patches of white downy growth on the surface of every culture. The two most striking properties all members of this group have in common are reduction in conidium number and an ability to grow in areas already occupied by the parent mycelium. There is wide variation among these mutants in conidium production, such that a whole spectrum of types is found, ranging from those which quite closely resemble the parent strain to the highly pleomorphic type which produces few or no conidia. All efforts, admittedly of a preliminary character, to demonstrate that these mutants are autoinduced and in some way related to the age of the parent cell or colony have led to failure or inconclusive results. No data have been found which are incompatible with application to this phenomenon of the concepts of spontaneous mutation plus positive selection. The method of classifying strains on the basis of conidium production is unsatisfactory because of the extreme sensitivity of this character to changes in the environment. In a subsequent paper the results of growth studies will reveal a simpler quantitative method for classification.

ACKNOWLEDGMENTS

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LITERATURE CITED

Acton, H. W., and N. C. Dey. 1934. A simple method of recovering typical cultures of dermatophytes from pleomorphic growths. Indian Med. Gaz. **59**: 601–604.

Bistis, G. N. 1957. A double-dish method of cultivating fungi. Mycologia **49**: 772–773.

Bodin, E. 1895. Les teignes tondantes du cheval et leurs inoculations humaines. Thèse de Paris.

Brown, W. 1926. Studies on the genus *Fusarium*. IV. On the occurrence of saltations. Ann. Bot. **40**: 223–243.

Cantanei, A. 1929. Sur les altérations des cultures d'un *Trichophyton gyseum* au cours du vieillissement. C. R. Soc. Biol. **102**: 807–808.

Chin, B., and S. G. Knight. 1957. Growth of *Trichophyton mentagrophytes* and *Trichophyton rubrum* in increased carbon dioxide tensions. Jour. Gen. Microb. **16**: 642–646.

Davidson, A. M., and P. H. Gregory. 1934. *In situ* cultures of dermatophytes. Can. Jour. Res. **10**: 373–393.

Emmons, C. W. 1931. Observations on *Achorion gypseum*. Mycologia **23**: 87–95.

——. 1932. Pleomorphism and variation in the dermatophytes. Arch. Derm. Syph. **25**: 987–1001.

Fox, T. C., and F. R. Blaxall. 1896. An inquiry into the plurality of fungi causing ringworm in human beings as met with in London. Brit. Jour. Derm. **8**: 241–255, 291–308, 337–359, 377–384.

Georg, Lucille K. 1954. The relationship between the downy and granular forms of *Trichophyton mentagrophytes*. Jour. Invest. Derm. **23**: 123–141.

Gregory, P. H. 1935. The dermatophytes. Biol. Rev. **10**: 208–233.

Grigoraki, Leon. 1925. Recherches cytologiques et taxonomiques sur les dermatophytes et quelques autres champignons parasites. Ann. Sci. Nat. Bot. X. **7**: 165–444.

——. 1956. Les champignons parasites des teignes. Mycopath. et Myc. Appl. **7**: 291–320.

Langeron, M., and S. Milochevitch. 1937. Sur une méthode employeé par Acton et Dey pour régénérer les cultures pléomorphisées des dermatophytes. Ann. Parasit. **15**: 177–181.

McVeigh, Ilda, and Florence Cambell. 1950. The growth of *Trichophyton mentagrophytes*. Mycologia **42**: 451–469.

Robbins, W. J., and Roberta Ma. 1945. Growth factors for *Trichophyton mentagrophytes*. Am. Jour. Bot. **32**: 509–532.

Robbins, W. J., and Ilda McVeigh. 1949. The "dual phenomenon" and *Trichophyton mentagrophytes*. Mycologia **41**: 128–140.

Sabouraud, R. Les Teignes. Masson et Cie, Paris, 1910.

——. 1929. Généralités concernant les dermatophytes. Le problème du pléomorphisme des cultures des dermatophytes. Ann. Derm. Syph. VI. **10**: 481–486.

Stockdale, Phyllis M. 1953. Requirements for the growth and sporulation of *Trichophyton persicolor*. Jour. Gen. Microb. **8**: 434–441.

Tate, P. 1929. The dermatophytes or ringworm fungi. Biol. Rev. **4**: 41–75.

Wilhelm, Stephan. 1947. The dual phenomenon in the dermatophytes. Mycologia **39**: 716–724.

AN ISOLATE OF FUSARIUM ROSEUM FROM HUMAN BURNS

JOHN E. PETERSON AND THOMAS J. BAKER[1]

(WITH 5 FIGURES)

A five-year-old boy, severely burned in a gasoline fire, was brought into the University of Missouri Hospital in August, 1957. Although the prognosis for the patient was poor, he not only survived but recovered to the point where initial skin grafting could be performed considerably sooner than is usually the case. The explanation of this statement concerns the fungus discussed in this paper.

CLINICAL OBSERVATIONS

The patient was treated by the exposure technique, which essentially consists of placing the patient on sterile sheets and leaving the burns open to the air. Nine days after injury the majority of the burns were covered with a white, cottony fungal growth. FIG. 1 is a photograph taken only three days after injury; even at this early date the fungal growth is quite apparent on the wounds. It is hypothesized that the fungus was introduced into the wounds when the patient's father rolled him directly on the earth in putting out the fire. This hypothesis is substantiated by the fact that the same fungus has been readily isolated from soil samples taken from the area where the boy was rolled.

Surgical debridement (removal of the crusts) was completed on the 15th day, as compared with the usual average of about 28–30 days. The junior author, who was the surgeon in charge of the case, is of the opinion that the fungus was responsible for the early separation of the crusts covering the burn surfaces. Entire sections of crusty debris could be readily lifted off without resort to the usual cutting and snipping. It appeared to the junior author that the fungus was producing a proteolytic substance which had freed the eschar from the underlying healthy tissues.

The fungus, isolated from a section of the eschar removed from the wounds, did not sporulate initially. However, when transferred onto

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a variety of media and onto agar-block slide cultures it soon produced the distinctive macroconidia of the genus *Fusarium*. There were now two reasons for more than a passing interest in this particular organism: first, the apparent therapeutic effect on the burns and the potential implications of this effect; second, the finding of a *Fusarium* sp., usually a plant parasite or a soil saprophyte or both, on human tissue.

RECORDS OF FUSARIUM SPP. ON ANIMAL TISSUES

Wollenweber and Reinking (**1935**) list only 22 of the approximately 145 species and varieties of *Fusarium* treated as separate entities as having been reported on animal tissues. All of these were found on such lower animals as nematodes, insects, arachnids, amphibians and reptiles with the exception of two, which were reported on birds and on a dog. The authors are aware of three reports of a *Fusarium* sp. being isolated from human tissue, all well since the publication of Wollenweber and Reinking's treatise. In all three instances the organism was apparently pathogenic.

Dr. Lucille Georg of the Communicable Disease Center, Chamblee, Georgia, has told the senior author that a *Fusarium* sp. was repeatedly isolated from the spinal fluid of a child with meningitis symptoms at the Columbia University Medical School some years ago. This report is unpublished. Sigtenhorst and Gingrich (**1957**) report a human corneal infection in which one of the etiological agents was a *Fusarium* sp. Mikami and Stemmermann (**1958**) describe a second case of human corneal infection in which the etiological agent was *Fusarium oxysporum*. The present report appears to be the fourth record of a *Fusarium* sp. on human tissue. In contrast to the preceding three, no pathogenesis is indicated in this case.

IDENTIFICATION AND DESCRIPTION OF THE FUNGUS

The organism isolated from the burns proved to be extremely variable in culture; sectoring was common and striking (FIG. 2). Single-spore isolations resulted in morphologically constant lines which were grown repeatedly on six media commonly utilized in studies of the genus *Fusarium*—potato plugs, steamed rice, 2% dextrose PDA, 5% dextrose PDA, lima-bean agar, and bean-pod agar. The resultant cultures were studied microscopically and all morphological characteristics were tabulated.

No microconidia were found. The macroconidia possessed a short, but distinct, foot and were produced on rounded, bulbous branches (FIG.

3). Chlamydospores were present in profusion in most cultures; they were generally intercalary (FIG. 4), but were occasionally found occurring terminally. Both sporodochia and sclerotia were produced under certain of the cultural conditions, as seen in FIG. 5 which also shows the general microscopic appearance of the organism and the total of the characteristics.

When the above characteristics are used to enter the keys in Wollenweber and Reinking (**1935**) and in the report of the 1924 Wisconsin

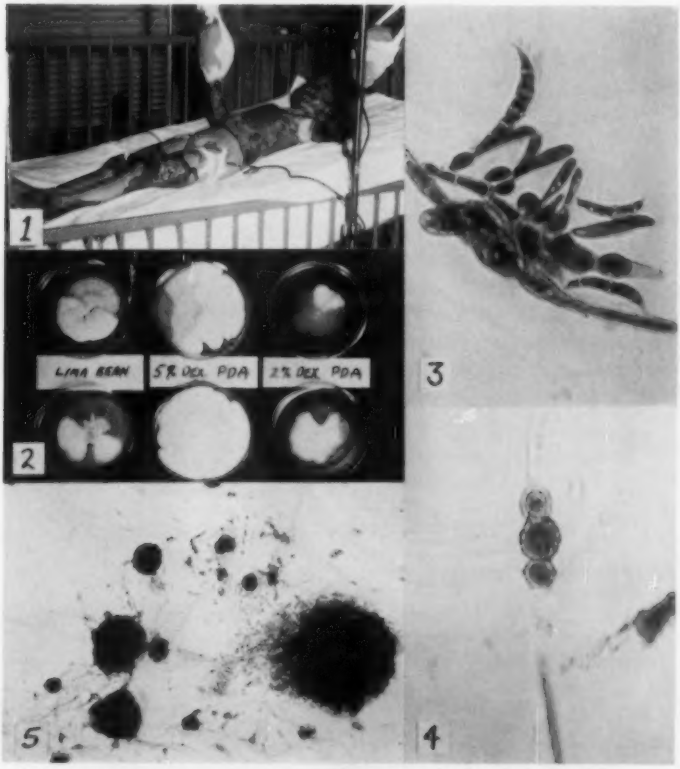

FIG. 1. Patient three days after suffering burns; fungus appearing as white patches on burned areas. FIGS. 2-5. *Fusarium roseum*, isolated from burns. FIG. 2. Pure cultures, showing sectoring and variation. FIG. 3. Macroconidia and mode of macroconidial production, × 1080. FIG. 4. Chlamydospores, × 1080. FIG. 5. General microscopic appearance, showing mycelium, macroconidia, chlamydospores, sclerotia, and young sporodochia.

Fusarium Conference (Wollenweber, et al., **1925**) this organism falls somewhere between Sections *Arthrosporiella, Gibbosum* and *Discolor*. There need be no quandary as to which of these sections of the genus *Fusarium* this particular isolate should be assigned to, because Snyder and Hansen (**1945**) grouped these three sections and Section *Roseum* together into one of their nine species. This organism isolated from human burns, then, has been determined to be an isolate of *Fusarium roseum* Link emend. Snyder & Hansen.

Studies of the proteolytic properties of this *Fusarium roseum* from human burns and of various other *Fusarium* spp. are now under way. In addition, the tremendous variation of the original isolate is being studied. Finally, the therapeutic possibilities of the organism and its products are being explored, not only by the authors, but also by a number of agencies interested in burn therapy and in drug production.

SUMMARY

An isolate of *Fusarium roseum* Link emend. Snyder & Hansen was found growing on the wounds of a severely burned child. Clinically, this was of interest because the fungus appeared to be instrumental in early separation of the burn eschar. Mycologically, the occurrence was of interest because members of the genus *Fusarium* have been known to occur on human tissue only rarely. Both clinical and mycological studies of this fungus and its products are in progress.

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LITERATURE CITED

Mikami, R. and G. N. Stemmermann. 1958. Keratomycosis caused by *Fusarium oxysporum*. Am. Jour. Clin. Path. **29**: 257–262.

Snyder, W. C. and H. N. Hansen. 1945. The species concept in *Fusarium* with reference to Discolor and other sections. Am. Jour. Bot. **32**: 657–666.

Sigtenhorst, M. L. and W. D. Gingrich. 1957. Bacteriologic studies of keratitis. South. Med. Jour. **50**: 346–350.

Wollenweber, H. W., C. D. Sherbakoff, O. A. Reinking, Helen Johann, and Alice A. Bailey. 1925. Fundamentals for taxonomic studies of *Fusarium*. Jour. Ag. Res. **30**: 833–843.

Wollenweber, H. W. and O. A. Reinking. 1935. Die Fusarien. Paul Parey, Berlin.

THE PEZIZACEAE OF THE MUSSOORIE HILLS (INDIA)—VII

K. S. Thind, Edith K. Cash, and Pritam Singh

(with 17 figures)

This paper records additional species of the Pezizaceae from the Mussoorie Hills (5000–7500 ft altitude in the northwestern Himalayas) as a part of the study of the fungus flora of that region undertaken by K. S. Thind and his students. The first six contributions (listed under references) describe thirty-one previously known species, one known variety, three new species and three new varieties. This seventh contribution deals with four more new species belonging to *Lamprospora, Humaria, Ascophanus* and *Humarina*. The fruit bodies are described from fresh material as well as from that preserved in alcohol-formalin.

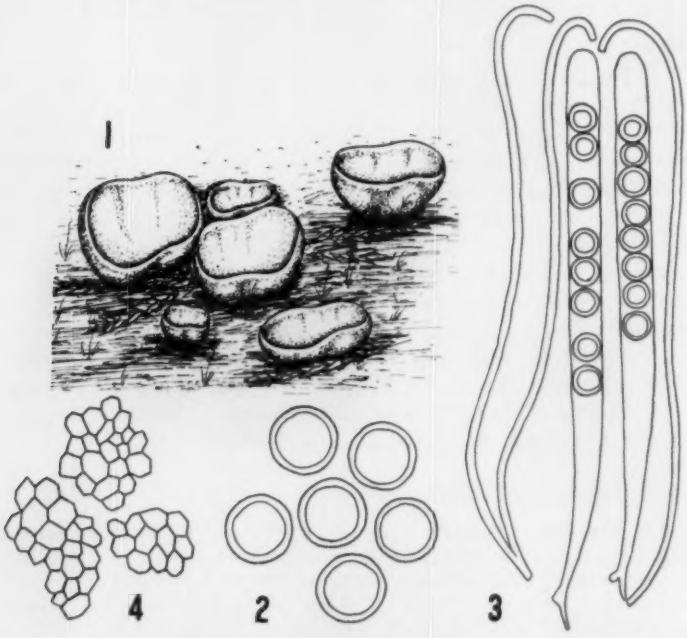
The numbers of the species are the serial numbers of the senior author's pezizoid flora of the Mussoorie Hills. Type collections have been deposited in the Herbarium of the Panjab University. Duplicate material is in the National Fungus Collections, Beltsville, Maryland.

39. **Lamprospora mussooriensis** sp. nov. Figs. 1–4

Apothecia usque 1–5 mm in diam., singula vel congesta, globosa dein patellata, saepe irregularia, cremea usque flavida, molle carnosa, sessilia, extus pallidioria; *asci* cylindrici, ad apices rotundati vel truncati, ad bases attenuati, ope iodi non caerulescentes, operculati, 180–213 × 9–13.5 μ; *ascosporae* 8, uniseriatae, hyalinae usque subhyalinae, globosae, glabrae, uniguttulatae, 9–11.2 μ in diam.; *paraphyses* subhyalinae, ad apices valde curvatae, 178–218 × 0.7–2 μ.

Apothecia up to 1–5 mm in diameter, single or densely gregarious to crowded, at first globose and closed, later becoming very shallow cupulate and finally discoid, sometimes with convex hymenial surface, regular but often becoming irregular and contorted from mutual compression, cream-colored to yellow, soft and fleshy, smooth, sessile, attached to the substratum at the base with the periphery free; external surface light yellow, slightly lighter colored than the hymenium, even, smooth; excipular cells subhyaline to dirty white (palish white) individually and very light brown (earth-colored) in mass, mostly polygonal (hexagonal), thin-walled, 5.4–14.4 μ wide; margin mostly entire, smooth, wavy in con-

torted specimens, incurved in young apothecia; hymenium yellow, slightly deeper colored than the external surface, even, smooth, plane to convex, concave in young apothecia. *Asci* cylindric, apex rounded to subtruncate or truncate, tapering gradually into a somewhat long stem-like base, not staining with iodine, operculate, 180–213 × 9–13.5 μ. *Ascospores* 8, uniseriate, overlapping in some asci, hyaline to subhyaline, wall darker, globose, smooth, uniguttulate, guttule large and filling three fourths of



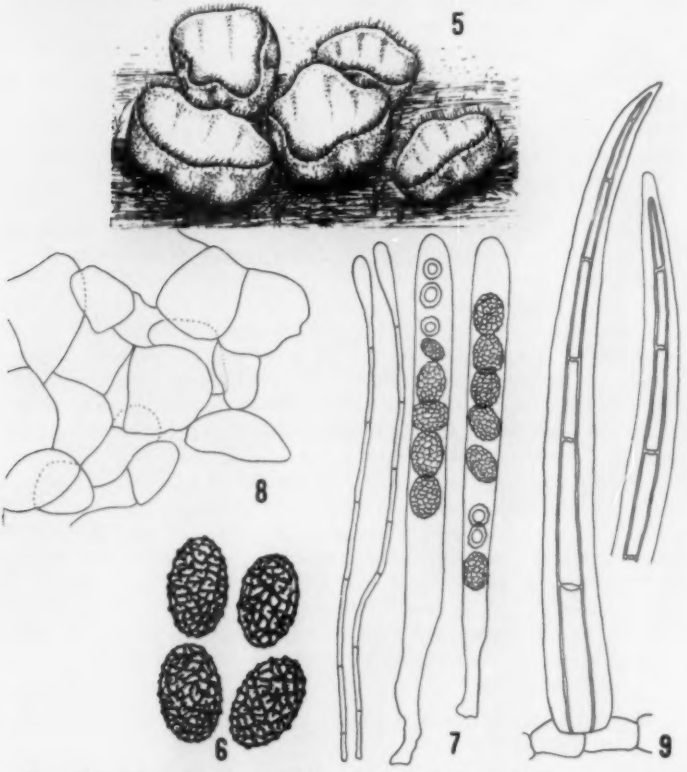
FIGS. 1–4. *Lamprospora mussooriensis*. FIG. 1, apothecia, × 5. FIG. 2, ascospores, × 950. FIG. 3, asci and paraphyses, and FIG. 4, excipular cells, × 400.

the spore cavity, 9.7–11.2 μ in diameter. *Paraphyses* pale ochraceous or subhyaline individually and yellowish brown in mass, filiform, nonseptate, simple or branched, apex not swollen but strongly curved, 178–218 × 0.7–2 μ.

Collected on soil amid mosses: India (Brewery Road, Mussoorie), August 12, 1956, *306*, TYPE.

This fungus is allied to some smooth-spored *Lamprospora* species. It comes close to *L. haematostigma* (Hedw.) Boud. but differs from it in having larger apothecia, and smaller and uniformly uniguttulate asco-

spores. It also differs from *L. carbonaria* (Fckl.) Seaver in having yellow apothecia and smaller ascospores, and from *L. haematostigma* (Hedw.) Boud. var. *gigantea* Thind & Singh in having yellow apothecia and uniformly uniguttulate ascospores.



FIGS. 5–9. *Humaria subreticulata*. FIG. 5, apothecia, × 5. FIG. 6, ascospores, × 950. FIG. 7, asci and paraphyses. FIG. 8, excipular cells, and FIG. 9, hairs, × 400.

40. **Humaria subreticulata** sp. nov. FIGS. 5–9

Apothecia 1.5–12 mm in diam., dispersa, gregaria vel interdum congesta et contorta, molle carnosa, sessilia, intense aurantiaca, extus pallidioria, pilis nigro-brunneis, simplicibus, rectis, rigidis, septatis, acutis, 187–981 × 16.2–34.2 μ dense obsita; *asci* cylindrici, ad apices rotundati vel interdum subtruncati, ad bases attenuati, ope iodi non caerulescentes, operculati, 187–234 × 10.8–18 μ; *ascosporae* uniseriatae, hyalinae, late ellipsoideae, verrucosae usque subreticulatae, uniguttulatae, 15–17.3

× 9.8–11.3 μ; *paraphyses* auranticae, clavatae, septatae, simplices, granulosae, 216–259 × 1.7–3.6 μ, ad apices usque 5.4–9 μ inflatae.

Apothecia 1.5–12 mm in diameter, scattered to gregarious, also often densely crowded, globose when young, mostly shallow cupulate, occasionally discoid, sometimes contorted from mutual compression, mostly even, deep orange, sometimes orange red, soft and fleshy, hairy, sessile, mostly spreading and prostrate, sometimes submerged slightly in the substratum; external surface orange, lighter colored than the hymenium, densely hairy; hairs dark brown under the low power of the microscope, but appearing almost black to the unaided eye, single, simple, conspicuous and long, erect, rigid and bristle-like, septate, wall very much thickened (up to 7.2 μ), apex acute and straight, base often forked, hairs shorter on the external surface and much longer and denser at the margin, forming a blackish fringe around the apothecium, 187–981 × 16.2–34.2 μ; excipular cells subhyaline, spherical to polygonal or variously angled and becoming irregular, pseudoparenchymatous, thin-walled, 21.6–67 μ wide; margin densely hairy, hairs longer and more conspicuous than on the external surface and forming a fringe around the margin of the apothecium, entire, becoming wavy in contorted apothecia; hymenium deep orange or orange red, deeper colored than the external surface, even, smooth, concave to plane. *Asci* cylindric, apex rounded to subtruncate in some cases, tapering below into a long or short stem-like base, not staining with iodine, operculate, 187–234 × 10.8–18 μ. *Ascospores* 8, 2–4 spores often degenerating and dwarfed, uniseriate, parallel to oblique, overlapping at the ends, hyaline, broadly ellipsoid, thick-walled, rounded at the ends, verrucose with warts arranged in lines and sometimes forming irregular ridges which give a subreticulate appearance, uniguttulate, guttule large and filling three fourths to almost the entire spore cavity, guttule more conspicuous in younger spores, 15–17.3 × 9.8–11.3 μ. *Paraphyses* orange, clavate, septate, simple, granular, orange colored and strongly enlarged at the tip, 216–259 × 1.8–3.6 μ, 5.4–9 μ wide at the tip.

Collected on soil amid mosses, India (Municipal Garden, Mussoorie), August 10, 1956, n. *307*, TYPE.

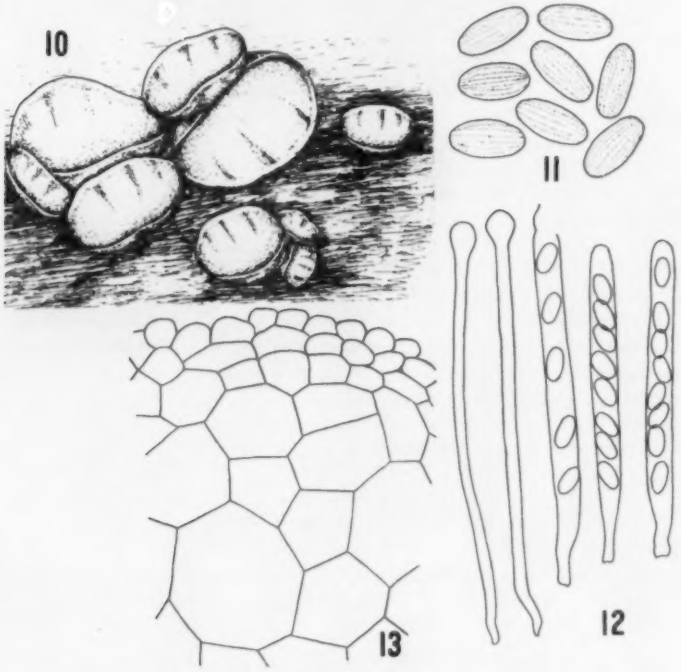
The spore ornamentation in this Mussoorie fungus suggests that found in species of *Melastiza*, but it appears to be closer to *Humaria* Fckl. Although the warts on the spore wall are arranged in irregular lines giving a subreticulate appearance, there is no definite network present as in spores of *Melastiza* species. It seems best, therefore, to place the fungus in *Humaria*.

41. **Ascophanus striatus** sp. nov. FIGS. 10–13

Apothecia 0.7–4 mm in diam., gregaria vel congesta, patellata, interdum contorta, aurantio-flavida, molle carnosa, glabra, sessilia, margine interdum undulata;

asci longe cylindrici, ad apices rotundati, ope iodi non caerulescentes, operculati, 124–162 × 9–10.8 μ; *ascosporae* 8, uniseriatae, hyalinae, glabrae, longitudinaliter indistincte striatae, 11.3–13.5 × 6–6.7 μ, paraphyses clavatae, aurantiacae, septatae, 133–166 × 4.5–6.3 μ, ad apices valde abrupteque usque ad 9–14.5 μ inflatae.

Apothecia 0.7–4 mm in diameter, densely gregarious to crowded, discoid, regular, sometimes contorted from mutual compression, orange yellow, soft and fleshy, smooth, sessile, attached to the substratum at the



FIGS. 10–13. *Ascophanus striatus.* FIG. 10, apothecia, × 10. FIG. 11, ascospores, × 950. FIG. 12, asci and paraphyses, and FIG. 13, excipular cells, × 400.

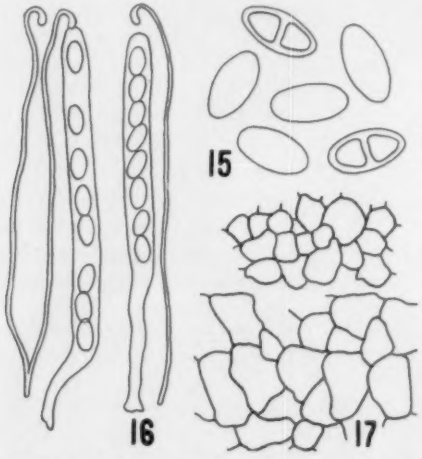
base with the periphery free; external surface orange yellow, smooth, excipular cells orange, polygonal, rounded along the margin, pseudoparenchymatous, thin-walled, 27–73 μ wide; margin smooth, even or somewhat wavy; hymenium orange yellow, concolorous with the external surface, smooth, plane. *Asci* cylindric-elongate, apex rounded, tapering below into a short stem-like base, not turning blue with iodine, operculate, 124–162 × 9–10.8 μ. *Ascospores* 8, uniseriate, usually oblique, ends overlapping, hyaline, wall dark, mostly narrow ellipsoid, ends

FIG. 14. *Humarina indica*, apothecia, × 2.

rounded, smooth, marked by faint longitudinal striations or narrow bands (about a dozen in number), non-guttate, 11.3–13.5 × 6–6.7 μ. *Paraphyses* clavate, orange, simple, nonseptate, strongly and abruptly enlarged at the tip, 133–166 × 4.5–6.3 μ, 9–14.4 μ wide at the tip.

Collected on cow dung: India (Dhanolti, Mussoorie), August 23, 1956, n. *308*, TYPE.

This species shows close relationship with *A. granulatus* (Bull.) Speg. but its spores are much smaller and marked by longitudinal striations or bands. In view of these two important differences this Mussoorie fungus is considered here as a new species. This, perhaps, is



FIGS. 15–17. *Humarina indica*. FIG. 15, ascospores, × 950. FIG. 16, asci and and paraphyses, and FIG. 17, excipular cells, × 400.

the only species of *Ascophanus* showing longitudinal striations or bands on the ascospores and is therefore named *Ascophanus striatus*.

42. **Humarina indica** sp. nov. FIGS. 14–17

Apothecia 2.5–9 mm in diam., 2–5.5 mm in altidudine, gregaria vel conferta, cupulata usque patellata, substipitata, nigro-brunnea, carnosa, delicater pilosa vel furfuracea, pilis simplicibus, septatis, hyalinis, 19.8–90 × 3.6–7.2 μ, margine dentato vel crenato, hymenio nigro-brunneo, stipite brevi, 0.7–3 × 1–2.3 mm; *asci* cylindrici, ad apices rotundati vel subtruncati, infra attenuati, ope iodi non caerulescentes, operculati, 135–180 × 7.2–10.8 μ; *ascosporae* 8, uniseriatae, hyalinae, ellipsoideae, glabrae, 9.7–12.7 × 6–7.5 μ; *paraphyses* subhyalinae vel ochraceae, filiformes, simplices vel ad bases ramosae, 164–198 × 1.8–3.6 μ, ad apices hamatae et usque ad 3.6–6.3 μ inflatae.

Apothecia 2.5–9 mm in diameter, and 2–5.5 mm in height, gregarious to crowded, cupulate when young and shallow cupulate to discoid when mature, regular, irregular or contorted from mutual compression, substipitate, dark brown, almost black and more or less retaining shape on drying, fleshy, delicately hairy; external surface brown, rough, delicately hairy or furfuraceous-verrucose; hairs delicate, simple, septate, flexuous, apex rounded, hyaline, 19.8–90 × 3.6–7.2 μ; excipular cells spherical to variously angled and irregular, light brown to brown, pseudoparenchymatous, thin-walled, 7.2–21.6 μ wide; margin delicately hairy, entire to dentate or crenate; hymenium dark brown, smooth, concave to plane; stipe very short, concolorous with the external surface, cylindric, expanding above into the apothecium, 0.7–3 × 1–2.3 mm. *Asci* cylindric, apex rounded to subtruncate, tapering below into a stem-like base, not staining with iodine, operculate, 135–180 × 7.2–10.8 μ. *Ascospores* 8, uniseriate, parallel to oblique, ends overlapping, hyaline, ellipsoid, ends obtusely rounded, smooth, biguttulate when young, non-guttate when mature, 9.7–12.7 × 6–7.5 μ. *Paraphyses* subhyaline to ochraceous individually, brown in mass, filiform, nonseptate, simple or branched at the base, enlarged and conspicuously hooked at the tip, 164–198 × 1.8–3.6 μ, 3.6–6.3 μ wide at the tip.

Collected on soil amid mosses: India (Kanatal, Mussoorie), August 22, 1956, n. *309*, TYPE.

The fungus appears to belong to the genus *Humarina* but does not seem to fit under any of its species reported so far. The substipitate apothecia are an unusual feature.

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REFERENCES

Seaver, F. J. 1928. The North American Cup-fungi (Operculates). Publ. by the author. pp. 1–284.

——. 1942. The North American Cup-fungi (Operculates). Supplement. Publ. by the author. pp. 285–377.

Thind, K. S. and L. R. Batra. 1957. The Pezizaceae of the Mussoorie Hills—I. Jour. Indian Bot. Soc. **36**: 51–60.

—— **and J. S. Sethi.** 1957. The Pezizaceae of the Mussoorie Hills—II. Jour. Indian Bot. Soc. **36**: 196–206.

—— **and** ——. 1957. The Pezizaceae of the Mussoorie Hills—III. Indian Phytopath. **10**: 26–37.

—— **and L. R. Batra.** 1957. The Pezizaceae of the Mussoorie Hills—IV. Jour. Indian Bot. Soc. **36**: 428–438.

——, **Edith K. Cash and J. S. Sethi.** 1957 [1958]. The Pezizaceae of the Mussoorie Hills—V. Mycologia **49**: 831–836.

—— **and Pritam Singh.** 1958. The Pezizaceae of the Mussoorie Hills—VI. Jour. Indian Bot. Soc. (in press).

CULTURAL BASIS FOR MAINTAINING POLYPORUS CINNABARINUS AND POLYPORUS SANGUINEUS AS TWO DISTINCT SPECIES

HAZEL H. MCKAY

(WITH 2 FIGURES)

INTRODUCTION

The sporophores of *Polyporus sanguineus* L. ex Fr. and *Polyporus cinnabarinus* Jacq. ex Fr. are sufficiently alike to be considered one species by many mycologists. Weir (**12**) suggests that *Polyporus sanguineus* might be the tropical form of *Polyporus cinnabarinus*, with climatic conditions responsible for the slight morphological differences. Cleland and Cheel (**2**), in their study of Australian fungi, point out the similarity of the two and state that, although *P. sanguineus* is considered to be a tropical species and *P. cinnabarinus* a temperate one, they both occur in the vicinity of Sydney. Lloyd (**6**) speaks of *P. cinnabarinus* as the temperate region analogue of *P. sanguineus*. Cunningham (**3**), in his study of the two, states that "there is no constant feature upon which the two species may be maintained; consequently collections should be merged under *Coriolus sanguineus* since this species precedes *Polyporus cinnabarinus* on the same page of the Systema."

In 1952 Bose (**1**), after studying the two in culture concluded that he was dealing with two distinct species. He pointed out differences in cultural characteristics, especially the pigmented granules on the hyphal walls of *P. sanguineus* and the lack of such granules in cultures of *P. cinnabarinus* except in the colored poroid areas where color develops with the basidia initials. When dicaryotic cultures were paired no hyphal fusions occurred between the two and a distinct line of aversion developed between their mats.

Matters (**7**), after working with cultures of the *P. cinnabarinus* complex in the southern hemisphere, notes the slower growth rate of *P. cinnabarinus* and the absence of hyphal incrustations but concludes that these represent different forms within a group, which do not merit specific rank.

Because of the confusion which exists, it seemed advisable, in the present study, to pair monosporous cultures of *P. sanguineus* and *P. cinnabarinus* and apply the clamp-connection criterion for the identity of species. Interfertility tests, using monosporous cultures from different sporophores for the identification of species among the heterothallic Hymenomycetes, have been used by various authors. The results of these tests are summarized by Nobles (**8**), Whitehouse (**13**) and Raper (**10**). If monosporous cultures, which are completely lacking in clamps, are obtained from two sporophores whose identity is in question and are paired, the production of clamps in the resulting culture indicates that

TABLE I

LIST OF CULTURES EXAMINED AND THEIR SOURCES

Culture and Herbarium Number	Host	Date of collection	Location
FP 103199	Sapling of *Myrica cerifera* L.	8/11/52	Baker Co., Fla.
FP 103548	Dead stem of *Campsis radicans* (L.) Seem.	11/8/52	St. Tammany Parish, La.
FP 103663	Felled tree of *Liquidambar styraciflua* L.	12/6/52	Isle of Wight Co., Va.
FP 103983	Down pine slash, *Pinus elliotti* var. *elliotti* Little	1/8/52	Jacksonville, Fla.
FP 103985	Down log of *Quercus laurifolia* Michx.	1/8/52	Jacksonville, Fla.
T-39	*Liriodendron tulipifera* L.	Summer '55	Morgantown, W. Va.
FP 103506	Fallen trunk of *Carpinus caroliniana* Walt.	11/5/52	Harrison Co., Miss.
FP 104131	Dead twig of *Castanea mollissima* Blume	9/8/52	Glen Dale, Md.
FP 103897	Hardwood slash	5/25/53	Ammendale, Md.
Ft. Belvoir	Unidentified	11/ /46	Ft. Belvoir, Va.
St. Thomas 1348	Dead leguminous wood	5/16/56	Virgin Islands
LCF 441	*Prunus avium* L.	11/24/42	Argentina
LCF 580	*Prunus persica* (L.) Batsch	3/ /48	Argentina
FP 104138	Hardwood log	9/13/56	Beltsville, Md.

the sporophores are of the same species. Conversely, in heterothallic fungi, it is accepted as strong evidence that two sporophores are of different species if their monosporous cultures fail to produce clamps when paired.

MATERIALS AND METHODS

The monosporous cultures were obtained either from freshly collected sporophores or from sporophores produced by polysporus cultures maintained in the Forest Disease Laboratory Fungus Collection at Beltsville, Maryland, and identified as *P. cinnabarinus* or *P. sanguineus*. Isolates were made from sporophores collected in eleven different localities from Maryland south to the Gulf States, two from Argentina and one from the Virgin Islands (TABLE I).

To obtain single-spore cultures a spore print was deposited on the

surface of a malt-agar plate by attaching a fragment of a mature sporophore to the inner face of a Petri-dish lid so that the pores opened toward and perpendicular to the agar. By means of a miniature spatula, spores from the spore print on the surface of the agar were streaked across a very thin layer of corn meal agar in another Petri-dish. The spores germinated in 24–36 hours. Under a dissecting microscope, the germinating spores in an area of proper dilution along the streaks were picked out singly and transferred to a tube of diamalt agar medium. Before attempting pairing studies these monosporous cultures were checked for absence of clamps.

TABLE II

RESULTS OF PAIRING MONOSPOROUS MYCELIA FROM 8 SPOROPHORES OF POLYPORUS SANGUINEUS WITH MONOSPOROUS MYCELIA FROM 6 SPOROPHORES OF POLYPORUS CINNABARINUS.

		103506	103983	104131	103199	LCF 580	LCF 441	St. Thomas 1348	104548	103985	103663	103897	103138	Ft. Belvoir	T-39
Group I *Polyporus sanguineus*	103506		+	+	+	+	+	+	+	0	0	0	0	0	0
	103983	+		+	+	+	+	+	+	0	0	0	0	0	0
	104131	+	+		+	+	+	+	+	0	0	0	0	0	0
	103199	+	+	+		+	+	+	+	0	0	0	0	0	0
	LCF 580	+	+	+	+		+	+	+	0	0	0	0	0	0
	LCF 441	+	+	+	+	+		+	+	0	0	0	0	0	0
	St. Thomas 1348	+	+	+	+	+	+		+	0	0	0	0	0	0
	104548	+	+	+	+	+	+	+		0	0	0	0	0	0
Group II *Polyporus cinnabarinus*	103985	0	0	0	0	0	0	0	0		+	+	+	+	+
	103663	0	0	0	0	0	0	0	0	+		+	+	+	+
	103897	0	0	0	0	0	0	0	0	+	+		+	+	+
	104138	0	0	0	0	0	0	0	0	+	+	+		+	+
	Ft. Belvoir	0	0	0	0	0	0	0	0	+	+	+	+		+
	T-39	0	0	0	0	0	0	0	0	+	+	+	+	+	

All cultures were grown on Difco malt agar or Fleischmann's diamalt agar according to the methods described by Davidson, Campbell, and Vaughn (**5**) and incubated at 25° C. All growth-rate studies were made on Difco malt agar, but diamalt agar was used for the pairing studies and for stock cultures.

INTERFERTILITY STUDIES

Using a single-spore isolate from each sporophore, pairings were made in all possible combinations as shown in TABLE II. The presence of clamps, indicating interfertiliy, is indicated by the plus (+) sign and their absence and non-compatability by the zero (0) sign. The pairings

were made by placing mycelia from the mat of each component about 20 mm apart on the surface of the culture medium in Petri-dishes and allowing them to grow for 7–14 days, at which time the cultures were examined microscopically.

The monosporous cultures were divided into two groups, I and II, according to their pairing compatibility. The members of group I are interfertile with each other but not with members of group II, and likewise the members of group II pair with each other but not with members of group I. This indicates that each group represents a different species.[1]

The sporophores of *P. sanguineus* and *P. cinnabarinus* in the Forest Disease Laboratory Herbarium, from which the cultures originated, were examined and found to conform in appearance with the two compatibility groups. The sporophores of group I have a smooth upper surface strongly marked with conspicuous zonations and very thin margins while those of group II show a rough, ridged surface and a thicker margin. This is in accordance with Overholts (**9**) who, when describing *P. cinnabarinus* states, "The chief distinction between this species and *P. sanguineus*, its only close relative, is that in *P. cinnabarinus* the pileus is typically rugose or uneven because of unequal compacting of the tomentum over the surface, whereas in *P. sanguineus* the surface is smooth and even, as though seared with a hot iron and the pileus is very thin." The theoretical basis for such interfertility tests as those used in the identification of cultures, and methods of applying them, are fully discussed by Nobles (**8**), Raper (**10**) and Whitehouse (**13**). The presence of clamps in polysporous cultures and of simple septa in their monosporous cultures indicates that both *Polyporus sanguineus* and *P. cinnabarinus* are heterothallic. Then, according to the theory of multiple allelomorphs at the loci for heterothallism, fertile matings between monokaryotic mycelia of the monosporous cultures, which are entirely devoid of clamps, lead to the establishment of a dikaryon, with clamps, when two compatible monokaryotic mycelia are grown in close contact. This takes place when the monosporous mycelia from two sporophores of the same species from different sources are grown together, as shown in TABLE II, where results show a complete pairing of monokaryotic mycelia of *P. cinnabarinus* from different localities, and also a complete pairing of *P. sanguineus* monokaryotic mycelia among themselves, and a lack of pairing between the two species.

[1] While this work was in progress similar results with the above mentioned species have been reported in a personal communication from Dr. Mildred Nobles, Senior Mycologist, Botany and Plant Pathology Laboratory, Science Service, Department of Agriculture, Ottawa, Canada.

CHARACTERISTICS OF POLYSPOROUS CULTURES

Twenty-five polysporous cultures in the Forest Disease Laboratory culture collection named either *P. cinnabarinus* or *P. sanguineus* were grown on various media for comparison. These fell into two groups comparable to sporophore characteristics and pairing groups. The cultures were grown on Difco malt agar and 0.5 per cent tannic- and gallic-acid agar made according to the method described by Davidson, Campbell and Blaisdell (**4**) and Davidson, Campbell and Vaughn (**5**).

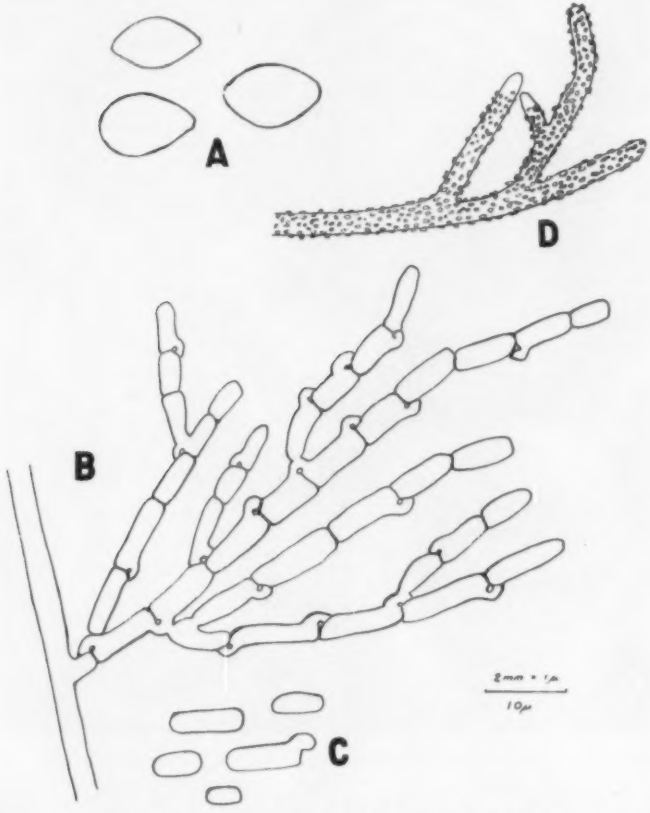

FIG. 1. *Polyporus sanguineus.* A. Chlamydospores. B. Fasciculate branches forming oidia. C. Oidia. D. Incrusted hypha.

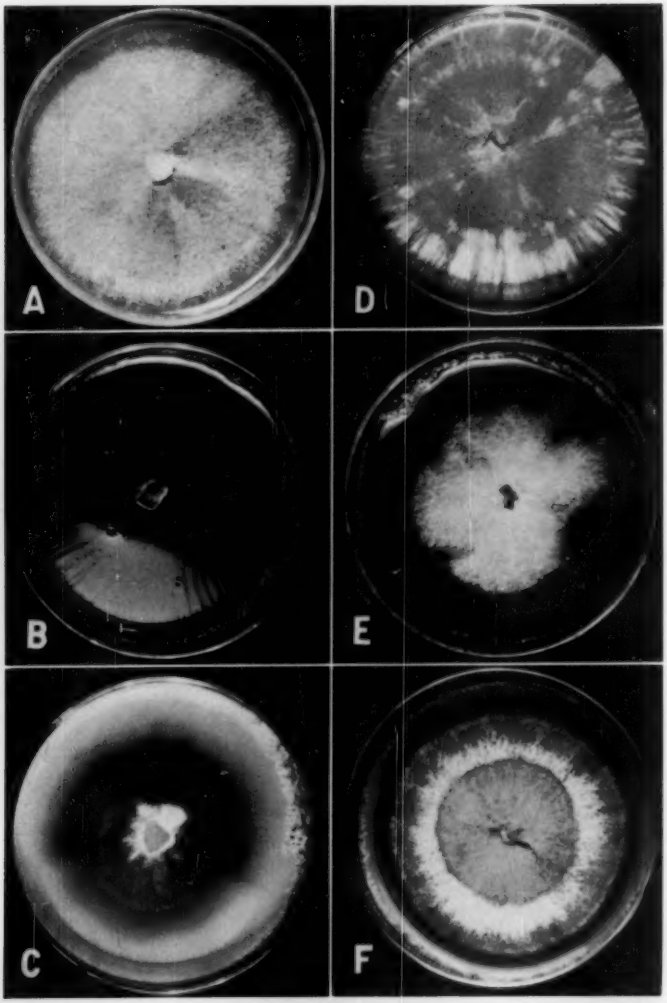

FIG. 2. Cultures grown for 14 days at 25° C in 90 mm Petri dishes. A–C. *Polyporus cinnabarinus* (Ft. Belvoir). D–F. *Polyporus sanguineus* (St. Thomas 1348). A and D. Cultures on 2% Difco malt agar. D and E. Cultures on 2% Difco malt agar + 0.5% gallic acid. C and F. Cultures on 2% Difco malt + 0.5% tannic acid.

Polyporus cinnabarinus Jacq. ex Fr.
Key pattern.—(5) A-P-M-1-2-11 or E-P-I-1-2-11-14 (Fig. 2, A–C)

Polysporous cultures of this species have been described by Nobles (8) and since the cultures in our collection in group II fit well Dr. Nobles description they are not redescribed here.

Polyporus sanguineus L. ex Fr.
Key pattern.—E-P-I-1-2-4-11-14 (Fig. 2, D–F)

Growth characteristics.—Growth moderately rapid, forming a mat 65–85 mm in diameter in 7 days and filling the Petri-dish in less than 14 days, mats white at first, thin, felty or woolly with farinaceous patches, soon developing irregular shaped, thick, wooly "flesh ocher" (11) "coral red" or "light coral red" areas in the central or marginal zones. At 14 days the colored area may be limited to the central zone, to the marginal zone, or extend over the entire plate; advancing zone thin, downy, white; margin even, appressed; odor faintly fruity or absent; positive oxidase reaction with fair growth on gallic- and good growth on tannic-acid agar.

Pores bearing basidia common in 3–4 weeks in scattered areas or along the margin of the mat.

Hyphal characteristics.—Staining hyphae 1.5–4 μ in diameter with numerous simple clamps; non-staining hyphae 1.5–3 μ in diameter, hyaline, fibrous, thick-walled, without clamps; oidia abundant in 13–18 days, formed by the separation of short cells in fasciculate branches bearing numerous clamps, broadly ovate to cylindrical, measuring 1.5–3 $\mu \times$ 3–8 μ, occasionally with the arm of a clamp attached; chlamydospores few to many, terminal or intercalary, ovoid to lemon-shaped, with thin walls, 5–8.5 $\mu \times$ 7.5–12 μ; hyphae in colored areas encrusted with minute yellow-red crystals soluable in KOH (Fig. 1, A–D).

Test-tube cultures.—In 28 days mats on slant thin, woolly and compact to loose-cottony, frequently with irregular-shaped thin areas and thick highly colored patches.

Type of decay.—Causing a white rot of dead wood.

Comparison of polysporous cultures of P. cinnabarinus and P. sanguineus.—The mats of the eleven *P. cinnabarinus* cultures studied remain white for 14 days except in very limited potential fruiting areas, which become "ochraceous salmon" or "zinc orange." On the other hand, the *P. sanguineus* cultures develop color as early as 5 days and by 14 days the mat is largely "flesh ocher" or "coral red." In rate of growth *P. sanguineus* is more rapid on malt and tannic acid agar, while

on gallic acid agar *P. sanguineus* grows fairly well but *P. cinnabarinus* only a trace. In reaction *P. cinnabarinus* produces a darker zone on tannic acid agar than does *P. sanguineus*. By 12–18 days oidia occurred in all cultures of *P. sanguineus* but were never found in *P. cinnabarinus*. In the highly colored portions of the mat of both species hyphae covered with fine incrustations of minute yellow-red crystals readily soluble in 5% potassium hydroxide solution are found. These are more abundant in *P. sanguineus* cultures since, in this species, the greater portion of the mat is colored. In test-tube cultures the two are very similar except that those of *P. sanguineus* are usually more highly colored.

CONCLUSION

The evidence furnished by a study of *Polyporus cinnabarinus* and *Polyporus sanguineus* in culture and the results from pairing single spore cultures, coupled with the morphological differences in the sporophore, suggests that the two are distinct species.

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BIBLIOGRAPHY

1. **Bose, S. R.** 1952. Identity of *Polystictus* (= *Polyporus* = *Trametes*) *cinnabarinus* (Jacq.) Fr. with *Polystictus sanguineus* (L.) Fr. Nature [London] **170**: 1020.
2. **Cleland, J. Burton, and Edwin Cheel.** 1917. Notes on Australian Fungi, No. IV. *Polyporus, Fomes* and *Hexagona*. Jour. Royal Soc. of New South Wales **51**: 473–557.
3. **Cunningham, G. H.** 1950. Australian Polyporaceae in herbaria of the Royal Botanic Gardens Kew and British Museum of Natural History. Proc. Linn. Soc. New South Wales **75**: 240.
4. **Davidson, Ross W., W. A. Campbell and Dorothy J. Blaisdell.** 1938. The differentiation of wood-decaying fungi by their reaction on gallic or tannic acid medium. Jour. Agr. Res. **57**: 683–695.
5. ——, —— **and Dorothy Blaisdell Vaughn.** 1942. Fungi causing decay of living oaks in the eastern United States and their cultural identification. U. S. Dept. Agr. Tech. Bull. **785**: 1–65.
6. **Lloyd, C. G.** 1924. Mycological Writings **7** (Mycological Notes 72): 1270.
7. **Matters, C. Nan.** 1955. Morphological characters of *Trametes cinnabarinus* (Jacq.) Fr., *Polyporus cinnabarinus* (Jacq.) Fr., and *Polystictus sanguineus* (L.) Mey. Progress Rept. No. 5, Forest Products Laboratory, S. Melbourne. pp. 1–11.

8. **Nobles, Mildred K.** 1948. Studies in forest pathology. VI. Identification of cultures of wood-rotting fungi. Can. Jour. Res., C. **26**: 281–431.
9. **Overholts, Lee Oras.** 1953. (Prepared for publication by Josiah L. Lowe.) The Polyporaceae of the United States, Alaska and Canada. Univ. of Mich Press. pp. 379–381.
10. **Raper, J. R.** 1953. Tetrapolar sexuality. Quart. Rev. Biol. **28**: 233–259.
11. **Ridgway, Robert.** 1912. Color Standards and Color Nomenclature. 43 pp. Washington, D. C.
12. **Weir, James R.** 1919. Concerning the introduction into the United States of extra-limital wood destroying fungi. Mycologia **11**: 58–65.
13. **Whitehouse, H. L. K.** 1949. Multiple-allelomorph heterothallism in the fungi. New Phytologist **48**: 212–244.

NOTES AND BRIEF ARTICLES

Schizophyllum umbrinum Berkeley in culture

In the hope of extending the study of sexuality and genetics in *Schizophyllum* to species other than the ubiquitous *S. commune* Fr., numerous attempts have been made to obtain fresh material of the less common, geographically restricted forms (Linder, **1933**). Except for a single specimen of *S. umbrinum* Berk. from Brazil provided by Prof. G. W. Martin in 1956, all of our own extensive collections in this country and all of a world-wide collection (Raper, Krongelb, and Baxter, 1958) were monotonously assignable to *S. commune* (Wm. Bridge Cooke, personal communication). In October, 1958, arrangements were made for A. S. Rhoads, of Jacksonville, Florida, to collect whatever material of the genus could be found in Southern Florida from Miami to Key West. Along with numerous collections of *S. commune*, Rhoads provided fresh material of *S. umbrinum* from 21 different stations. Unfortunately, however, he was unable to find *S. fasciatum* Pat., known from this region only from a single collection (originally designated *S. alneum*) made in 1915 by J. K. Small on Old Rhodes Key, despite a few hours' search on this key.

Upon receipt of the collections of *S. umbrinum*, a few well-preserved fruit bodies of 15 different collections were suspended above agar medium; three to six days later, 11 of these had revived and were shedding spores, and from each was isolated a sample of 32 monosporous progeny. In culture, the white, fluffy vegetative mycelium is undistinguished; it grows well on the various media, including minimal medium, routinely used for *S. commune* (Raper and Krongelb, **1958**).

An extensive series of tests has revealed no evidence whatever of mycelial interaction, either heterokaryosis or dikaryosis. On "fruiting medium," monosporous cultures, intra-stock matings, and inter-stock matings produce fruit-body primordia in most cases within ten days. Further development to the stage of gilled fruiting bodies occurs in high and approximately equal frequency in single cultures and in matings of the two different kinds. The fruiting bodies produced in culture strongly resemble those found in nature and, beginning in the early primordial stages, are a bright orange in color with a tendency to change to a rich chocolate brown in advanced age. Hyphal fragments teased

from the stipe and trama of fruiting bodies showed no clamp connections, and only simple septa were found upon the hyphae of mycelia grown from such fragments.

The nuclear condition, as revealed by phase microscopy, is the same in monosporous mycelia, in mated mycelia, and in mycelia derived from fruiting bodies. In each case practically all cells were either mononucleate or binucleate, and these occurred with approximately equal frequency; trinucleate cells were very rarely seen. Most of the cells of

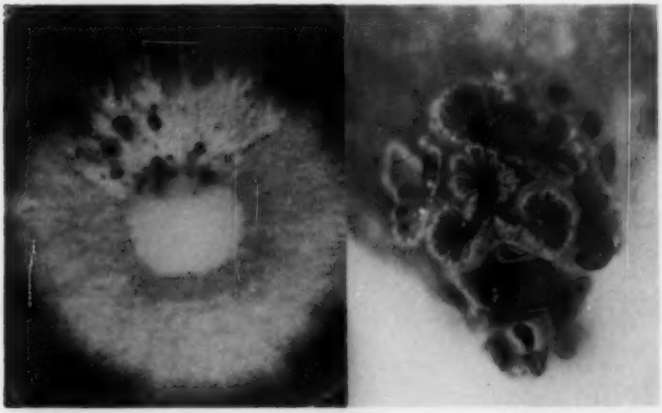

FIG. 1. *Schizophyllum umbrinum* Berkeley. On left, a mating between two monosporous cultures; no discernible interaction occurred between the mates, one of which has produced a number of small fruiting bodies. × 1. On right, a group of fruiting bodies with associated mucous droplets. × 4.

individual hyphae were either mononucleate or binucleate, but exceptions were not difficult to find. The nuclear condition thus provides no evidence of dikaryosis or other mycelial interaction.

No interaction could be found between monosporous mycelia of *S. umbrinum* and *S. commune*. That this isolation also operates in nature is indicated by two of Rhoads's collections, in each of which perfectly normal fruiting bodies of both species were intermixed upon the same piece of wood.

There was remarkably little variation in the original monosporous mycelia, and early subcultures yielded only mycelia like the originals, although there occurred occasional sectors that grew significantly faster than the mycelia from which they originated. More than half of 150

subcultures that were made from the originals after six weeks, however, were of an altered mycelial type comparable to the mutant *thin* of *S. commune:* fast growing, depressed habit with little or no aerial mycelium, failure to fruit, and the release of pigments (rarely including indigo) into the medium. Because of this marked and rapid degeneration of the vegetatively propagated mycelium, no cultures of *S. umbrinum* are being deposited in any of the culture collections.

The original collections of *S. umbrinum* have been turned over to Wm. Bridge Cooke, who has examined most of the collections of *Schizophyllum* with which we have worked, to distribute or to dispose of as he deems fitting.

The author wishes to express his indebtedness to Dr. Wm. Bridge Cooke for his taxonomic contributions to the continuing study of sexuality in *Schizophyllum* and especially to Dr. A. S. Rhoads, who would accept reimbursement only for actual and very modest expenses incurred on a week's collecting trip, for the materials of *S. umbrinum*.—JOHN R. RAPER, Harvard University.

LITERATURE CITED

Linder, D. H. 1933. The genus *Schizophyllum*. I. Species of the Western Hemisphere. Amer. Jour. Bot. **20**: 552–564.

Raper, J. R., Gladys S. Krongelb, and Margery G. Baxter. 1958. The number and distribution of incompatibility factors in *Schizophyllum*. Amer. Nat. **92**: 221–232.

—— **and** ——. 1958. Genetic and environmental aspects of fruiting in *Schizophyllum commune* Fr. Mycologia **50**: 707–741.

MANUSCRIPT

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